AD-A178 854 SEQUENTIAL MORPHOLOGIC ALTERATIONS IN THE FOVEOLA AND CORNEA OF NONHUMAN (U) PACIFIC MEDICAL CENTER SAN FRANCISCO CA W H SPENCER SEP 85 DAND17-79-C-9132 1/1

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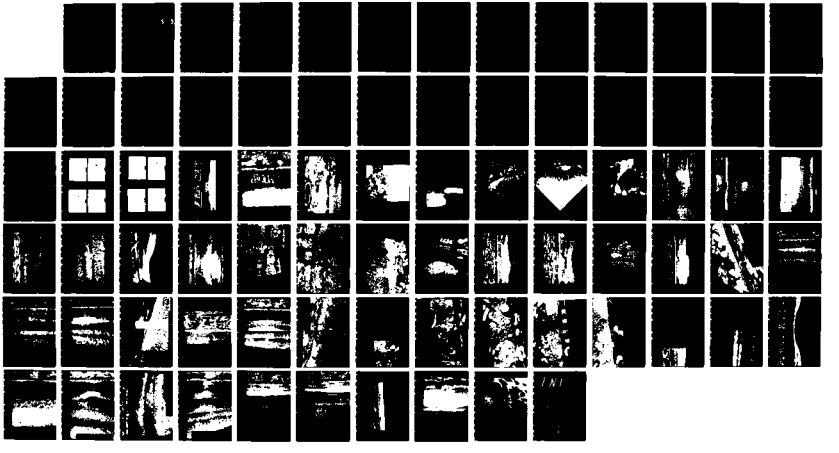

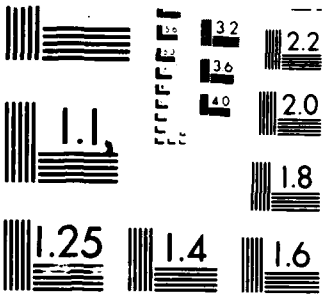

SEQUENTIAL MORPHOLOGIC ALTERATIONS IN THE FOVEOLA AND CORNEA OF NONHUMAN SUBJECTS AFTER EXPOSURE TO COHERENT LIGHT

Final Scientific Report

September, 1985

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Contract No. DAMD17-79-C-9132

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp Date Jun 30, 1986

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Pacific Presbyterian Medical Center 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) 2340 Clay Street San Francisco, California 94115	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-79-C-9132
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick, Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62777A; PROJECT NO. 3E1627. 772A878; TASK NO. BA; WORK UNIT ACCESSION NO 204

11. TITLE (Include Security Classification)
(U) Sequential Morphologic Alterations in the Foveola and Cornea of Nonhuman Subjects After Exposure to Coherent Light

12. PERSONAL AUTHOR(S)
William H. Spencer, M.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual/ Final Report*	FROM 10/1/79 TO 9/14/85	1985 September	52

16. SUPPLEMENTARY NOTATION
*Annual for the period Mar 85-Sep 85
Final for the period Oct 79-Sep 85

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
20	05		Q-switched ruby laser; Sequential clinical &
06	18		Frequency doubled neodymium laser; histopathological
			Foveolar retinal lesions; observations

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This is an investigation of the sequential (one hour, one day, one week, one month) clinical and morphologic (light and electron microscopic) effects upon the foveolar and parafoveolar retina of single short duration pulses of coherent light at 694.3 nm and 532 nm at total intraocular energy levels of approximately three times ED50. Focal injury to the retina and subjacent choroid is found to be less marked at 532 nm than at 694.3 nm. The lesions are sharply circumscribed and primarily involve the outer layers of the retina and the retinal pigment epithelium. Lesser damage occurs in the inner retina and in the choroid. Morphologic evidence of healing is noted within one hour, and progresses almost to completion within one month. The process involves a combination of phagocytosis, cellular migration and possibly cellular proliferation. Inflammatory cell infiltration and scarring is not observed.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Judy Pawlus	22b. TELEPHONE (Include Area Code) 301-663-7325 22c. OFFICE SYMBOL SGRD-RMI-S

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted
All other editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE

FOREWORD

In conducting the research described in this report the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Page Number

TABLE OF CONTENTS

TABLE OF CONTENTS

REPORT

This is a report of an investigation performed at the Eye Pathology Laboratory of the Pacific Presbyterian Medical Center (PPMC) as a joint effort with personnel of the Division of Biorheology at the Letterman Army Institute of Research (LAIR). The study is designed to obtain baseline comparative information regarding the sequence of morphologic events that occur in the foveolar and parafoveolar area of the cynomolgus monkey retina following exposure to single short duration pulses of coherent light at 694 nm (q-switched ruby laser) and at 532 nm (frequency doubled neodymium laser). Plans to follow this study with an investigation of the effects upon the cornea of focal coherent light at 10.6 microns (carbon dioxide laser) and an investigation of the potential beneficial effects of non-steroidal anti-inflammatory agents upon the extent of the laser induced retinal lesions have not been carried out because an animal use protocol for the studies was not obtained by LAIR.

BACKGROUND

In order to provide a base upon which safety standards may be established for humans, the Division of Biorheology at Lair has conducted a series of investigations in nonhuman subjects of the functional and morphologic effects of coherent and incoherent light upon ocular tissue. Mechanical, thermal and photochemical retinal damage is known to be produced by exposure to optic sources that produce a discrete image upon the retina at wavelengths between 400 and 1400 nm. The extent of tissue damage produced depends upon the energy level, its wavelength, and its duration of exposure. Significant loss of central visual acuity may occur after a single short pulse exposure of the central portion of the retina (foveola and immediate surround) to laser irradiation. Because of this, ophthalmologists who use lasers to therapeutically photocoagulate lesions in the retina carefully avoid this region. However, inadvertent foveolar and parafoveolar laser burns occasionally occur and these produce permanent injuries. Similar foveolar and parafoveolar burns may occur in the eyes of civilian or military personnel who are exposed to low energy laser systems either inadvertently or in battlefield situations. The extent of the retinal injury and the degree of visual loss varies with the diameter of the burn, the wavelength of the coherent light, the amplitude and duration

of the incident laser energy and the individual's pupil size. Retinal burns may potentially be produced by frequency doubled neodymium laser sources up to a distance of 3.2 kilometers from the subject. The potential for retinal injury is enhanced when the subject is using an optical device such as a telescope or binoculars, and in such situations injury to the retina may be produced by lasers up to 10.5 kilometers distant. The total intraocular energy (TIE) necessary to have a 50% probability of causing an ophthalmoscopically visible retinal response (ED50) has been calculated for lasers at varying wavelengths. Lasers currently used as military range finders and designators are capable of greatly exceeding ED50 dosage levels. The clinical sequence of events following photocoagulation of extrafoveal retina is well known but these events have not been documented with sequential morphologic (light and electron microscopic) observations nor have sequential studies been performed when the foveola is photocoagulated. Clinically within an hour after extrafoveolar photocoagulation, a white spot becomes visible. During the next three or four days the spot enlarges and appears edematous obscuring the underlying pigment epithelium. Over the next two to three weeks, the edema subsides permitting observation of the deeper retinal layers and the retinal pigment epithelium which exhibits focal loss of pigmentation with pigment migration. The extent of the definitive lesion is usually apparent within three to four weeks. In the study described below we have documented and compared the sequential morphologic alterations produced by focal exposure of the retina to coherent light at two different wavelengths (694 nm, 532 nm).

SCOPE OF WORK

Exposure Data:

Right Eye: The central retina (fovea and parafoveolar region) of the right eye of each of four cynomolgus monkeys was exposed to a pattern of five separated single short pulses (10 nanosecond) of Q-switched coherent light (ruby laser) at 694 nm. Each pulse was 50 microns in diameter and varied from 25 to 39 microjoules (figure 1). The total intraocular energy for each burn was approximately three times ED50. Each spot was designated as a single lesion (L). A series of surrounding larger marker burns (MB) was also placed for purposes of specimen orientation. The pattern is depicted in figure 2. Each marker burn was created with a single pulse of coherent light of the same wavelength, diameter and duration as the pulse used to create the lesional burns. However, the energy levels employed were larger and varied from 54 to 125 microjoules (figure 1). The lesional burns were barely visible with

direct ophthalmoscopy but the surrounding marker burns were readily seen (figure 3).

Left Eye: The central retina (fovea and parafoveolar region) of the left eye of each of the above-noted cynomolgus monkeys was exposed to a single short pulse (10 nanosecond) of frequency doubled neodymium coherent light (532 nm). Each pulse was 50 microns in diameter with energy levels varying from 6.8 to 8.5 microjoules (figure 1). The total intraocular energy for each lesion burn (L) was approximately three times ED50. As with the right eye, the lesional burns were placed in a pattern identical to that depicted in figure 2. Surrounding marker burns were then placed, each produced by a multiple pulse of the same wavelength, diameter and duration as each of the lesional burns and at a higher energy level (see figure 1). As with the right eye, the marker burns were readily seen with the direct ophthalmoscope but the central lesional burns could barely be discerned (figure 4).

Intervals from Exposure to Sacrifice, Tissue Fixation, Preparation and Sectioning: Animals were sacrificed at Letterman Army Institute of Research, one hour, one day, one week and one month after exposure. Fixation was obtained by intracardiac perfusion with Tyrodes nutrient buffer followed by "triple fix." (Triple fix is comprised of a mixture of 2.5% glutaraldehyde, 2.5% paraformaldehyde and 0.5% diflurodinitrobenzene in 0.1 molar cacodylate buffer.) This was augmented by intravitreal injection with 0.5 cc triple fix. In each animal the right eye was enucleated first followed within 15 minutes by enucleation of the left eye. The eyes were then transported to the Eye Pathology Laboratory at the Pacific Presbyterian Medical Center where the exposed portions of the retina together with adherent choroid and sclera were identified and removed en-bloc from the remainder of the eye under dissecting microscopic control. The blocks were trimmed to an average of 4 x 4 mm and then embedded in Epon for sectioning. Using an ultramicrotome, step serial 1 micron thick sections were cut through each block. The sections were numbered and as each lesional and marker burn was encountered, its location and identity was compared with the map of the pattern of exposures. As each lesion was located multiple thin electron microscopic sections were obtained and studied. The process was extremely time-consuming and required the preparation and evaluation of several thousand sections.

Evaluations of Sections: One micron and thin sections were compiled from each marker burn and each lesion in all eyes. The characteristic features of tissue alterations were identified and each layer was studied (choroid, Bruchs' membrane, retinal pigment epithelium, rod and cone outer and inner segments and nuclei, outer plexiform layer, inner portions of the retina). The alterations were then quantified with the microcomputer image analyzer and the area and volume of each retinal lesion recorded

Findings

1. A) Right Eye:

One Hour Marker Burns: It is to be noted that approximately one hour elapsed at Letterman Army Institute of Research between exposure of the right eye of each animal to coherent light at 694 nm and exposure of the left eye of each animal to coherent light at 532 nm. Thus, sacrifice occurred almost two hours after exposure of the right eye and one hour after exposure of the left eye. In effect the lesions in the right eye are one hour older than those in the left eye in each animal.

Choroid: There is evidence of injury to choroidal melanocytes in the mid stroma of the choroid resulting in cell breakdown and dispersion of pigment granules. A few burn lesions also exhibit evidence of vascular injury with collections of free red blood cells partially surrounding mid stromal venules (figure 5 A,B). Rarely fibrinoid necrosis of the choriocapillaris is seen (figure 6).

Bruchs' Membrane: No alterations are seen.

Retinal Pigment Epithelium: The retinal pigment epithelium (RPE) within the confines of the lesion is separated from Bruchs' membrane and most of the RPE cells within the lesion exhibit severe damage (figure 5 A,B). Undamaged RPE cells (figure 7) at the margins of the lesion exhibit extensions of their cytoplasm toward the center of the burn (figure 8 A,B). These changes are present in all burns and appear to be a form of early repair response. No macrophages are seen.

Rods and Cones: There is severe disruption of the outer and inner segments of the rods and cones in all burns. Cellular debris is dispersed over the entire extent of each lesion (figure 9). The affected rod and cone nuclei exhibit pyknosis and appear deeply stained.

Outer Plexiform Layer: Dark staining material is visible along the course of axons emanating from the pyknotic rod and cone nuclei (figure 10).

Inner Retina: These layers appear undamaged.

Parameters of the Lesions: The parameters of these lesions are listed in figure 33.

B) Right Eye:

One Hour Lesions:

Choroid: Occasional choroidal melanocytes exhibit cell membrane disruption with pigment granule dispersion. The choriocapillaris is intact with only occasional damage to endothelial cells. There is no evidence of choroidal hemorrhage.

Bruchs' Membrane: No alterations are seen.

Retinal Pigment Epithelium: The RPE cells within the confines of the lesion are separated from Bruchs' membrane and most of the cells exhibit severe damage (figure 11A,B). The undamaged RPE cells at the margins of the lesion have developed cellular extensions which remarkably in this short one hour interval since laser exposure have almost covered the retinal pigment epithelial defect (figure 11A,B).

Rods and Cones: The outer and inner segments of the rods and cones are severely disrupted. The affected nuclei exhibit pyknosis and appear deeply stained (figure 11A,B).

Outer Plexiform Layer: Occasional dark staining axons are identified and appear to be amanating from the pyknotic rod and cone nuclei (figure 12).

Inner Retina: These layers appear undamaged.

Parameters of the Lesions: The parameters of these lesions are listed in figure 33.

C) Right Eye:

One Day Marker Burns:

Choroid: The changes are identical to those seen in the one hour marker burn lesions (figure 13).

Bruchs' Membrane: No alterations are seen.

Retinal Pigment Epithelium: The damaged retinal pigment epithelial cells within the confines of the lesion are still present. The undamaged pigment epithelium to either side of the lesion has created cell extensions which almost cover the defect. No macrophages are identified.

Rods and Cones: Disrupted rods and cones are scattered throughout the lesion They resemble those seen in the one hour marker burn lesions. No macrophages are present. The affected nuclei of the rods and cones appear pyknotic.

Outer Plexiform Layer: Dark staining axons are observed in this layer. Several are traceable to the area of pyknotic nuclei in the outer nuclear layer (figure 14).

Parameters of the Lesions: The parameters of these lesions are listed in figure 33.

D) Right Eye:

One Day Lesions: The changes are virtually identical to those seen in the one hour lesions. Macrophages have not as yet appeared in the choroid nor in the region of the injured retinal pigment epithelial cells and rod and cone outer segments. The surrounding undamaged retinal pigment epithelial cells have almost covered the defect in the retinal pigment epithelium (figures 15A,B,C).

Parameters of the Lesions: The parameters of these lesions are listed in figure 33.

E) Right Eye:

One Week Marker Burns:

Choroid: Cells containing round, as well as spindle shaped granules, appear in the choroid for the first time. These are believed to be macrophages (figure 16). Fortuitous sections show cells partially passing between the retinal pigment epithelium and the choroid through Bruchs' membrane (figures 17A,B; 18A,B). These appear to be macrophages that have partially engulfed damaged retinal pigment epithelial pigment granules. Similar cells are not noted in the region of the destroyed retinal pigment epithelium. There is considerably less cellular debris with partial clearing of the destroyed outer and inner segments. The integrity of the retinal pigment epithelial layer has now been almost totally re-established by migration from undamaged RPE cells at the margins of the lesion. The covering RPE cells are less pigmented, thinner and possess a greater number of interdigitation processes than the surrounding normal RPE. A few pyknotic nuclei are seen in the outer nuclear layer. This is especially

apparent when the residual nuclei in the outer nuclear layer are compared with the adjacent undamaged retina (figure 19A,B).

Parameters of the Lesions: The parameters of these lesions are listed in figure 33.

F) Right Eye:

One Week Lesions: The changes are very similar to those noted in the marker burns, however, they are considerably less extensive. Macrophages are present which have cleared much of the debris from the destroyed retinal pigment epithelial cells and rod and cone outer segments. The retinal pigment epithelium has covered the defect and the basilar processes of the retinal pigment epithelium adjacent to Bruchs' membrane closely resemble those of the surrounding normal undamaged retinal pigment epithelial cells (figure 20).

Parameters of the Lesions: The parameters of these lesions are listed in figure 33.

G) Right Eye:

One Month Marker Burns: The lesions have essentially healed, however, the choroid still contains occasional macrophages. The retinal pigment epithelium appears to be entirely repaired but occasional macrophages are noted in the region of the disrupted rods and cones. Many of the outer segments of the rods and cones have regenerated. The nuclei of the rods and cones still appear depleted in number as compared with the surrounding undamaged rod and cone nuclei. Occasional pyknotic cells are seen (figure 21).

Parameters of the Lesions: The parameters of these lesions are listed in figure 33.

H) Right Eye:

One Month Lesions: These lesions are miniscule and are easy to overlook. They can be identified by the presence of occasional macrophages in the region of the retinal pigment epithelium and in the choroid. The RPE within the confines of the lesion appears somewhat thinner than the surrounding RPE (figures 22A,B).

II. A) Left Eye:

One Hour Marker Burns:

Choroid: Several of the marker burn lesions exhibit evidence of injury to mid stronal choroidal melanocytes resulting in dispersion of pigment granules. Some of the choriocapillary vessels contain scattered lymphocytes (figure 23).

Bruchs' Membrane: No alterations are seen.

Retinal Pigment Epithelium: The retinal pigment epithelium is disrupted within the confines of the lesion but not displaced away from Bruchs' membrane. The retinal pigment epithelial cells at the edge of the lesion exhibit cytoplasmic extensions toward its center. No macrophages are seen (figure 24).

Rods and Cones: There is outer segment disruption of these elements within the confines of the lesion. However, the inner segments appear intact and there is no pyknosis of the nuclei of the affected rods and cones. The degree of outer segment injury is much less than in the right eye (figure 25 and figure 26).

Outer Plexiform Layer: The outer plexiform layer is unremarkable and does not exhibit focal areas of darkened "streaks".

Inner Retina: These layers appear undamaged.

Parameters of the Lesions: The parameters of these lesions are listed in figure 33.

B) Left Eye:

One Hour Lesions:

Choroid: The choroid appears unaffected except for the presence of occasional disrupted melanocytic cells with associated pigment granule dispersion. The choriocapillaris appears undamaged. There is no evidence of choroidal hemorrhage (figures 27A,B).

Bruchs' Membrane: No alteration is seen.

Retinal Pigment Epithelium: RPE cells within the confines of the lesion are disrupted. Undamaged RPE cells at the margins of the lesion exhibit extensions of their cytoplasm toward the area of retinal pigment epithelial disruption (figures 27A,B).

Rods and Cones: There is disruption of the outer segments of the rods and cones in the area of the lesion. No pyknotic rod and cone nuclei are observed.

Outer Plexiform Layer: No abnormalities are noted in this layer.

Inner Retina: These layers appear undamaged.

Parameters of the Lesions: The parameters of these lesions are listed in figure 33.

C) Left Eye:

One Day Marker Burns:

Choroid: The changes in the choroid are identical to those seen in the one hour marker burn lesions. The disrupted retinal pigment epithelial cells and outer segments within the confines of the lesion are still present with no evidence of macrophage activity nor of clearing out of the debris. The retinal pigment epithelium to either side of the lesion has now almost completely covered the defect via cytoplasmic extension. There is outward bowing of the remaining layers of the retina with occasional pyknotic nuclei in the outer nuclear layer and dark-staining axons in the outer plexiform layer. The outer limiting membrane is partially disrupted (figures 28A,B).

D) Left Eye:

One Day Lesion: The changes are similar to those seen in the one hour lesions with no evidence of macrophages nor of clearing of the retinal pigment epithelial nor outer segment rod and cone debris. The undamaged retinal pigment epithelium is separated from Bruchs' membrane. The undamaged retinal pigment epithelial cells at the margins of the lesion have partially covered the defect in the retinal pigment epithelium. Several of the rod and cone inner segments appear pyknotic (figure 29).

E) Left Eye:

One Week Marker Burns: The choroid contains cells resembling macrophages which exhibit engulfed damaged retinal pigment epithelial pigment granules. Bruchs' membrane appears undamaged. The retinal pigment epithelial layer has been almost totally re-established by migrating cells from undamaged retinal pigment epithelial cells at the margins of the lesions (figure 30). The disrupted retinal pigment epithelial cells and outer segments seen in the one hour and one day lesions are now absent, however, occasionally bizarre retinal pigment epithelial cells and macrophages persist (figure 31). The outer nuclear layer contains only occasional pyknotic nuclei and the outer plexiform layer appears unremarkable. The inner retinal lyaers appear undamaged.

F) Left Eye:

One Week Lesions: These lesions appear well along in the healing process. The choroid appear unremarkable except for the presence of an occasional macrophage containing retinal pigment epithelial granules. Bruchs' membrane is intact and apparently normal. Partially depigmented retinal pigment epithelial

cells now cover the area of the original defect (figures 32A,B). Much of the disrupted rod and cone outer segments and degenerated retinal pigment epithelial cells and/or macrophages are also noted. In the foveolar region clear spaces are noted along the inner limiting membrane. These may represent degenerating nerve fibers or be a result of fixation artifact (figure 32C). However, they are limited to a very small area of the retina (figure 32A).

DISCUSSION

On the basis of this data, it would appear that retinal burns produced by exposure to coherent light at 694.3 nm (ruby laser - right eye) are more serious and require greater healing than those at 532 nm (frequency doubled neodymium laser - left eye). The dissimilarity may be attributed to the differences in wavelength of the coherent light and to the greater amount of incident energy used to produce the lesions in the right eye. The total intraocular energy (TIE) necessary to have a 50% probability of causing ophthalmoscopically visible retinal response (ED50) has been calculated for lasers at varying wavelengths. In this study lesional burns were produced at energy levels calculated to be approximately three times ED50. These lesions were barely visible with the ophthalmoscope. The marker burns, however, were more readily visible in each eye. These were produced at energy levels roughly three times the levels used to produce the lesion in the right eye. In the left eye higher energy levels in either single or multiple pulses were used to produce the marker burns. These were readily visible with the ophthalmoscope but the lesional burns produced with a single lower energy pulse were very difficult to discern. Clearly, there is a direct correlation between the level of the incident energy and the severity of the lesions.

Our data is also convincing in comparing the severity of the burns on the basis of wavelength differences. As noted in figure 33 the area and volume of the one hour lesions is considerably smaller in the left eye exposed to wavelength 532 nm (frequency doubled neodymium laser) than the area and volume of the one hour lesion in the right eye exposed to wavelength 694.3 nm (ruby laser). We have chosen to only compare the one hour lesions since these are the only ones that have sharply defined margins that permit accurate measurement. Migration of retinal pigment epithelial cells toward the center of the lesion proceeds rapidly; by one day the thinned marginal retinal pigment epithelial cells have migrated into the lesion causing obscuration of the borders of the burn. It is difficult to determine whether a thinned cell at the edge of a burn represents a damaged retinal pigment epithelial cell at the true margin of the burn or merely a thinned undamaged surrounding cell.

The diameter of the one hour lesions in each eye are approximately the same, averaging 90 microns in the right eye and 82 microns in the left eye. This is to be expected since the diameter of the exposure was the same in each eye (50 micron spot size). The primary reason for the increased area and volume of the right eye (ruby laser) lesions appear to be that the choroid exhibits greater evidence of injury (pigment dispersion and hemorrhage) than in the left eye (frequency doubled neodymium laser). The left eye lesions also appear to show less change to the inner segment of the rods and cones. Thus the lesions in the right eye have greater depth than those in the left eye.

The nature of the injury in each of the tissues studied and the sequence of events during the healing process is similar in all burns. Choroidal injury is more evident in the right eye and at higher energy levels; it is manifested by scattered melanocytic breakdown and occasional minor degrees of hemorrhage. Choroidal healing is rapid and appears to be effected in part by macrophages derived from the circulation. We have not observed phagocytosis of dispersed pigment or blood cells by resident choroidal cells, but this mechanism cannot be ruled out. Only occasional lymphocytes were seen in the choroidal vessels or in the overlying retina and it seems reasonable to conclude that an inflammatory cell infiltration is not a significant component of the response to this form of injury.

Bruchs' membrane shows no evidence of injury in any of our sections, however, increased permeability to cells may have been produced. Observation of macrophage-like cells passing through Bruchs' membrane in two burns (figures 17A,B; 18A,B) suggests this possibility. Sections adjacent to these sites show no evidence of injury. It is not known whether similar passage of macrophages through Bruchs' membrane can occur outside the area of injury.

The most severely affected tissues are the retinal pigment epithelium and the rod and cone outer segments. The disrupted cells and cellular elements appear to be removed primarily by phagocytes of undetermined origin. The phagocytic cells resemble retinal pigment epithelial cells, however, their source is unclear. These apparently seem to have the ability to migrate through Bruchs' membrane into the choroid bypassing the choriocapillaris en-route. This pathway for removal of degenerated cellular elements is not normally seen in the retina. In normal human and primate retinas the outer segments of the rods and cones are phagocytized and digested by normal retinal

pigment epithelial cells. Large particulate material tends to accumulate between the retinal pigment epithelium and Bruchs' membrane. Cellular passage (depicted in figures 17 A,B and 18 A,B) appears to occur as a reaction to injury as a second pathway. The thinned retinal pigment epithelial cells covering the original defect show no evidence of phagocytic activity in our sections.

Within the lesion, the rapidity by which cells at the margin of the burn produce cellular extensions and migrate toward the center of the lesion is impressive. Within one hour cytoplasmic extensions of marginal undamaged retinal pigment epithelial cells is apparent. In the small burns, almost complete coverage of the damaged area occurs within one week. Reparative retinal pigment epithelial cells are relatively non-pigmented and somewhat thinner than those in the surrounding undamaged retinal pigment epithelium, however, their basal laminar attachments to Bruchs' membrane appear reasonably normal. There is no evidence of metaplasia of the retinal pigment epithelial cells toward a fibroblast-like cell nor is there evidence of fibroblastic proliferation in the area of injury.

The destroyed rod and cone outer and inner segments appear to be removed by a process of phagocytosis. In most burns the debris has been removed within one week and regenerated outer segments seem to form within one month. The pyknosis and increased density of staining of the nuclei of the affected rods and cones suggests that the injury affects the entire neuronal cell and not merely its outer segment. The presence of dark staining material along the course of the axon as it passes through the outer plexiform layer is further evidence of injury affecting all parts of this neuronal cell. It is presumed that the regenerated outer segments seen in the one month burns represent regeneration derived from non-pyknotic cells.

We have not found evidence of trans-neuronal degeneration, however, in the one week lesions in the left eye, clear spaces are noted along the region of the inner limiting membrane. These may represent degenerating nerve fibers or more likely be the result of fixation artifact.

CONCLUSIONS

This investigation clearly shows that there is a direct correlation between the level of incident energy and the severity of the lesions produced. The study also demonstrates that lesions produced at 532 nm (frequency doubled neodymium) are less severe than those at 694.3 nm (ruby laser). The lesions produced in the left eye at 532 nm are not only less severe but they also appear to be more rapidly and completely repaired than those produced in the right eye at 694.3 nm. This is seen throughout the sequence of sections and is most apparent at one week where there is still readily detectable histologic evidence of injury in the right eye lesions but in the left eye the lesions are almost healed. At one month the right eye lesions are still detectable but the left eye lesions are not. It is interesting that lesions are also not evident in the photograph of the one month left eye provided to us by LAIR (figure 4).

The lesions produced at both wavelengths primarily affect the retinal pigment epithelium and outer retina. Only a minimum inflammatory response is elicited. This is characterized mainly by phagocytosis. Repair is not accompanied by fibroblastic proliferation in any of the injury sites. It begins almost immediately and is accomplished by cellular migration of retinal pigment epithelial cells from immediate surrounding cell population. This is accompanied by regeneration of rod and cone outer segments from residual viable cells in the outer nuclear layer. Cellular debris in the region of the rod and cone outer segments and destroyed retinal pigment epithelial cells is phagocytized by migrating cells. Their source is not apparent. It is possible that these cells originate in the choroid and migrate into the subretinal space where they phagocytize debris with subsequent passage back into the choroid. We have not observed evidence that they arise from the surrounding retinal pigment epithelial cells, however, they bear a resemblance to pigment epithelial cells and the pigment granules in their cytoplasm are football shaped. We cannot ascertain whether or not these granules are membrane bound.

We have not observed evidence of regeneration of rod and cone nuclei. Damaged cells in the outer nuclear layer undergo pyknosis and degeneration. A large number of cells in this layer appear to be undamaged and these probably serve as the source for regeneration of new outer segment material. The functional capacity of the repaired injury sites is not known.

FIGURES

FIGURE 1 A - ONE HOUR (CYNOMOLGUS #7)

RIGHT EYE (694.3 nm)		LEFT EYE (532 nm)	
BURN NUMBER	TIE (μJ)	BURN NUMBER	TIE (μJ)
MB 1	95.3	MB 1	
MB 2	89.8	MB 2	
MB 3	101	MB 3	
MB 4	91.1	MB 4	Each Burn = 50 pulses at 9.5 μJ/pulse
MB 5	106	MB 5	
MB 6	79.1	MB 6	
MB 7	84.2	MB 7	
MB 8	84.0	MB 8	
MB 9	105	MB 9	
L 1	32.2	L 1	9.07
L 2	26.7	L 2	9.36
L 3	35.3	L 3	10.5
L 4	31.3	L 4	10.9
L 5	25.6	L 5	10.5

NOTE: MB = MARKER BURN
L = LESION
μJ = MICROJOULES
TIE = TOTAL INTRAOCULAR ENERGY

DATA PROVIDED BY LAIR

Figure 1 A,B,C,D

Tables designating the energy level for each marker burn and lesion in the right and left eyes of each of four cynomolgus monkeys. (data provided by LAIR)

FIGURE 1 B - ONE DAY (CYNOMOLGUS #2)

RIGHT EYE (694.3 nm)		LEFT EYE (532 nm)	
BURN NUMBER	TIE (μJ)	BURN NUMBER	TIE (μJ)
MB 1	54.5	MB 1	27.9
MB 2	77.0	MB 2	33.5
MB 3	71.9	MB 3	32.9
MB 4	64.5	MB 4	29.5
MB 5	62.4	MB 5	32.8
MB 6	59.7	MB 6	30.8
MB 7	77.9	MB 7	30.4
MB 8	70.9	MB 8	33.1
MB 9	70.1	MB 9	28.9
L 1	37.6	MB 10	31.7
L 2	32.7	L 1	7.97
L 3	28.9	L 2	8.45
L 4	33.6	L 3	7.49
L 5	37.7	L 4	8.45
		L 5	8.21

NOTE: MB = MARKER BURN
L = LESION
μJ = MICROJOULES
TIE = TOTAL INTRAOCULAR ENERGY

DATA PROVIDED BY LAIR

FIGURE 1 C - ONE WEEK (CYNOMOLGUS #8)

RIGHT EYE (694.3 nm)		LEFT EYE (532 nm)	
BURN NUMBER	TIE (μJ)	BURN NUMBER	TIE (μJ)
MB 1	110	MB 1	
MB 2	107	MB 2	
MB 3	107	MB 3	
MB 4	122	MB 4	
MB 5	111	MB 5	Each Burn = 100 pulses
MB 6	109	MB 6	at 10.7 μJ/pulse
MB 7	104	MB 7	
MB 8	113	MB 8	
MB 9	112	MB 9	
MB 10	114	MB 10	
MB 11	119	MB 11	
MB 12	125	MB 12	
MB 13	115	L 1	10.81
L 1	32.4	L 2	12.12
L 2	37.1	L 3	13.01
L 3	35.7	L 4	12.06
L 4	36.5	L 5	12.47

NOTE: MB = MARKER BURN

L = LESION

μJ = MICROJOULES

TIE = TOTAL INTRAOCULAR ENERGY

DATA PROVIDED BY LAIR

FIGURE 1 D - ONE MONTH (CYNOMOLGUS #3)

RIGHT EYE (694.3 nm)		LEFT EYE (532 nm)	
BURN NUMBER	TIE (μJ)	BURN NUMBER	TIE (μJ)
MB 1	73.9	MB 1	
MB 2	77.2	MB 2	
MB 3	76.3	MB 3	
MB 4	85.7	MB 4	Each Burn = 50 pulses at 9.9 μJ/pulse
MB 5	75.4	MB 5	
MB 6	68.7	MB 6	
MB 7	72.5	MB 7	
MB 9	69.4	MB 9	
L 1	27.7	L 1	11.4
L 2	31.6	L 2	10.1
L 3	26.7	L 3	9.26
L 4	29.5	L 4	9.95
L 5	30.3	L 5	9.11

NOTE: MB = MARKER BURN
L = LESION
μJ = MICROJOULES
TIE = TOTAL INTRAOCULAR ENERGY

DATA PROVIDED BY LAIR

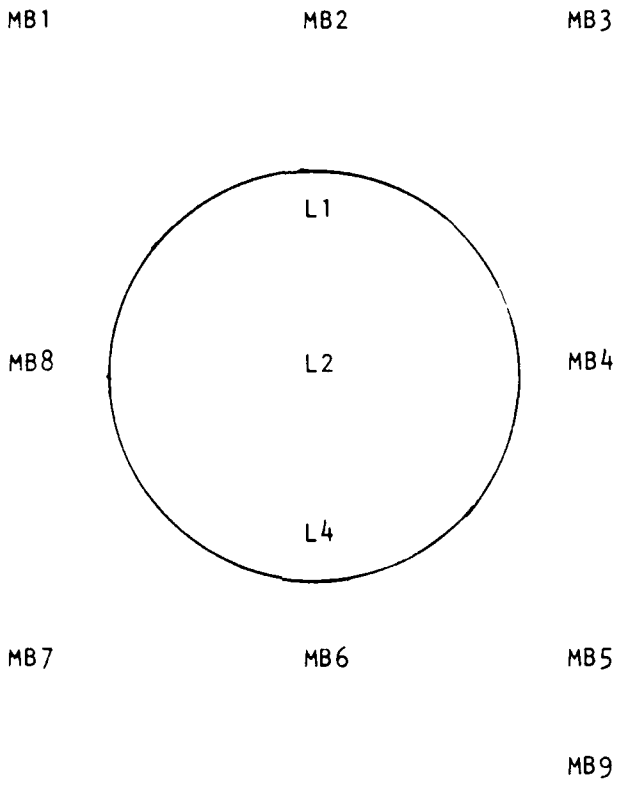

Figure 2

Diagram depicting the pattern of lesions and marker burns in the left and right eyes of each of the four animals. MB indicates site of marker burns; L indicates site of lesion. Adjacent numbers indicate order of exposure and correlate with numbers in figure 1.

FIGURE 33

RIGHT EYE (694.3 nm)

	ONE HOUR MARKER BURN	ONE HOUR LESION
AREA	12840 microns^2	6263 microns^2
VOLUME	1.163×10^6 microns^3	3.746×10^5 microns^3
DIAMETER	173.1 microns	90.13 microns

LEFT EYE (532 nm)

	ONE HOUR MARKER BURN	ONE HOUR LESION
AREA	10510 microns^2	3306 micron^2
VOLUME	1.154×10^6 microns^3	1.394×10^5 microns^3
DIAMETER	214.1 microns	82.5 microns

SEQUENTIAL MORPHOLOGIC ALTERATIONS IN THE FOVEOLA AND CORNEA OF NONHUMAN SUBJECTS AFTER EXPOSURE TO COHERENT LIGHT

Final Scientific Report

September, 1985

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Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-79-C-9132

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San Francisco, California 94115

RIGHT EYE

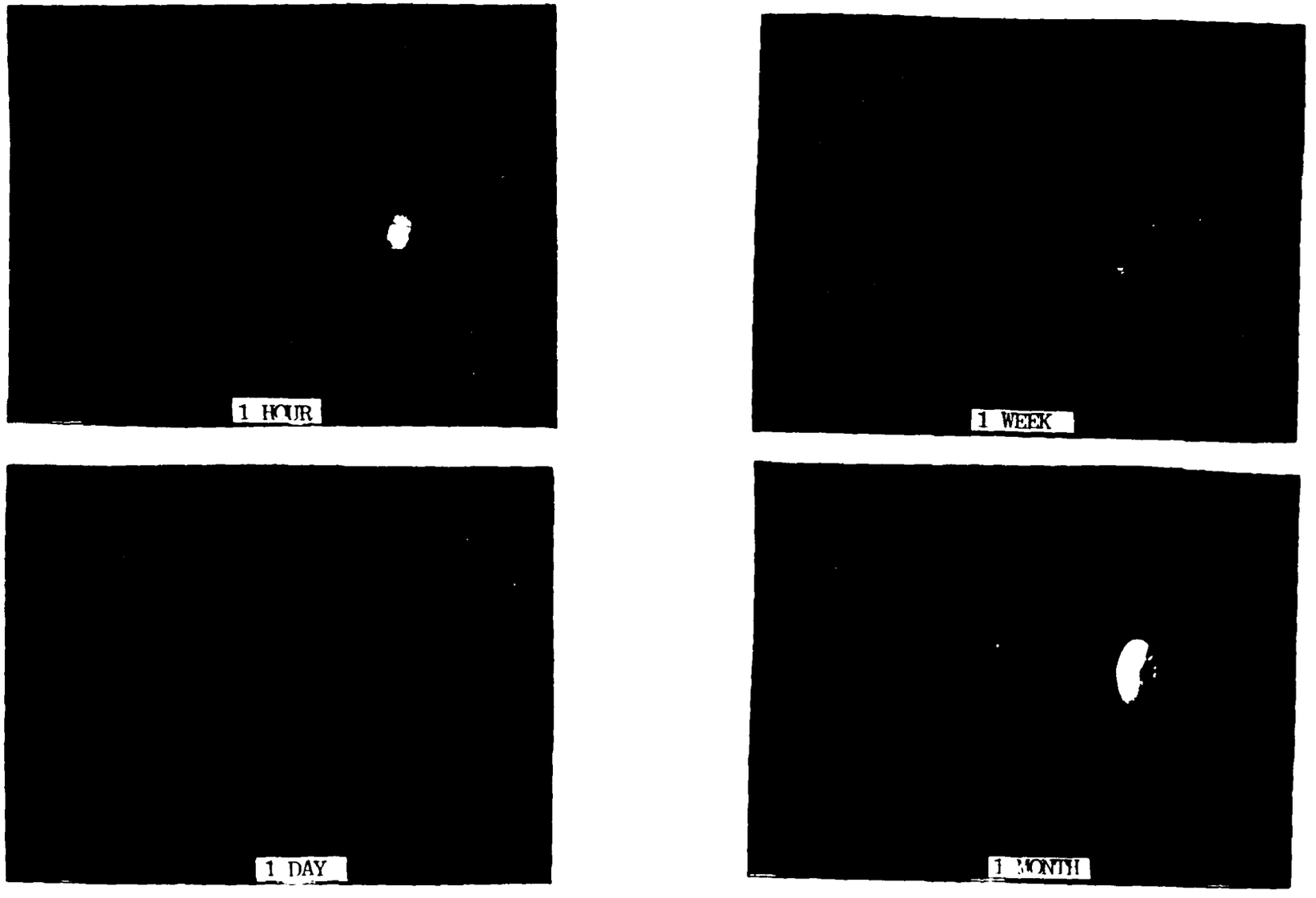


Figure 3

Polaroid photographs of the appearance of the central retina of the right eye of each of the four cynomolgus monkeys immediately after exposure to separated single short pulses of Q-switched coherent light at 694 nm. The surrounding marker burns are readily visible but the lesional burns

LEFT EYE

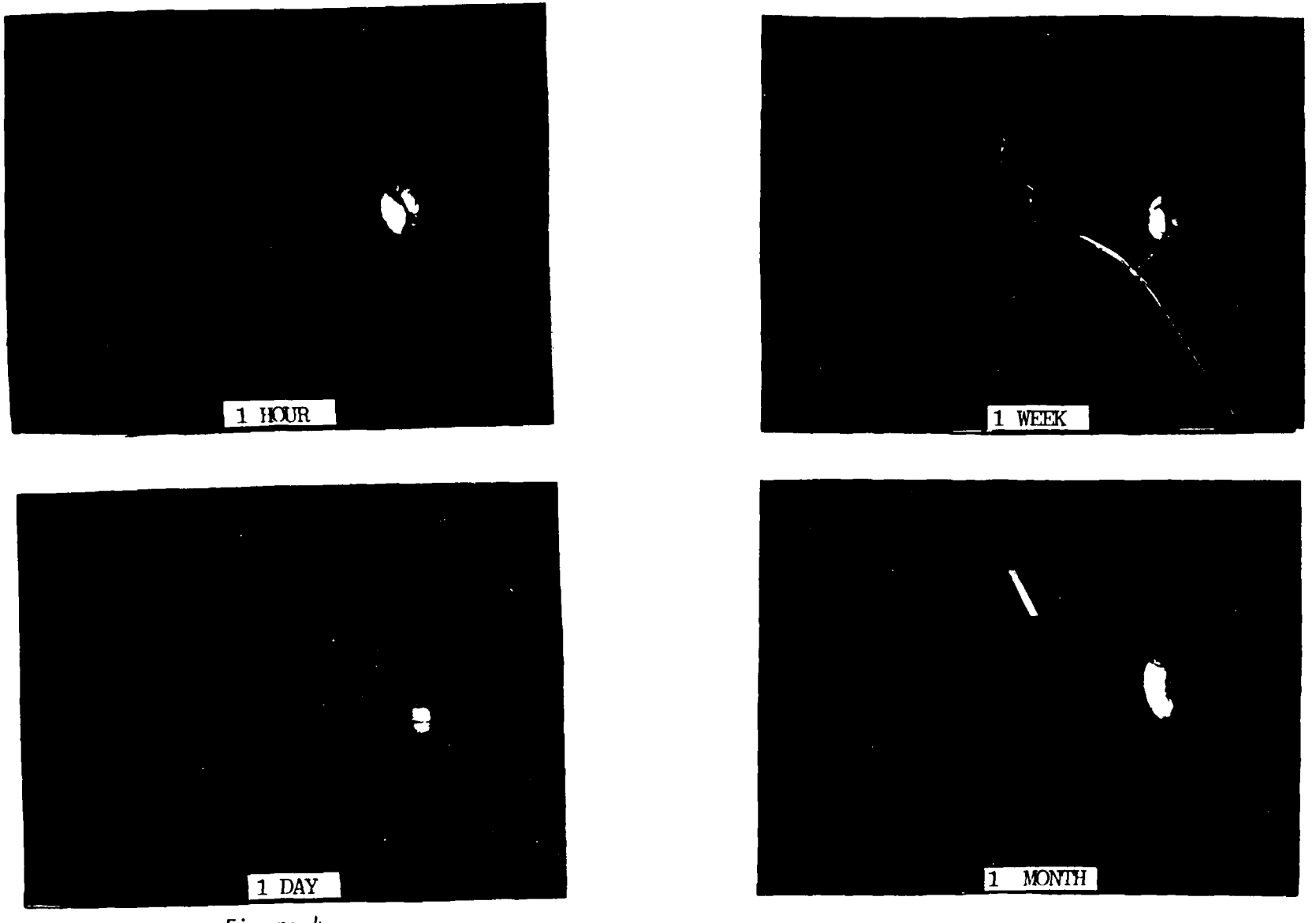


Figure 4

Polaroid photographs of the central retina of the left eye of each of four cynomolgus monkeys exposed to a single short pulse of frequency doubled neodymium coherent light at 532 nm. The marker burns are readily seen but

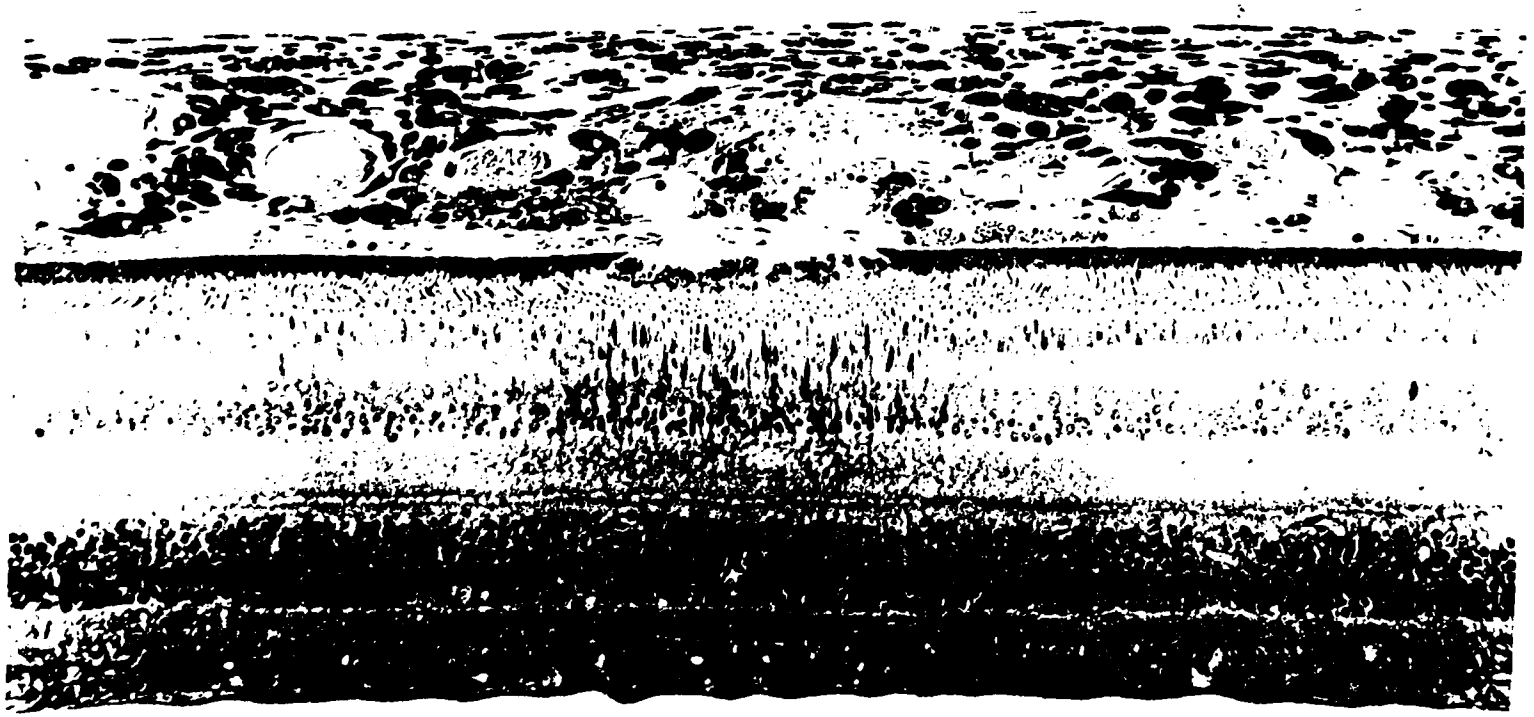


Figure 5 A - Right Eye

One hour marker burn. Low power view of lesion exhibiting intrachoroidal hemorrhage and dispersion of pigment granules within choroid. x 225

Figure 5 B

At slightly higher power free red blood cells and dispersed pigment granules within the choroid are more readily seen. x 560

Figure 6 A - Right Eye: One hour marker burn

Fibrinoid necrosis is observed in the choriocapillaris immediately subjacent to the area of retinal pigment epithelial disruption. x 900

Figure 6 B

A cell, presumed to be a macrophage, extends through the injured wall of a choroidal vessel. A structure resembling a pigment granule is seen in its cytoplasm. Additional dispersed pigment granules are seen toward the left side of the photograph. x 7500

Figure 7 - Right Eye: One hour marker burn

Bruchs' membrane is intact. The lower portion of the photograph depicts the interior of a capillary within the choriocapillaris in which there is a red blood cell and an acute inflammatory cell. x 16,500

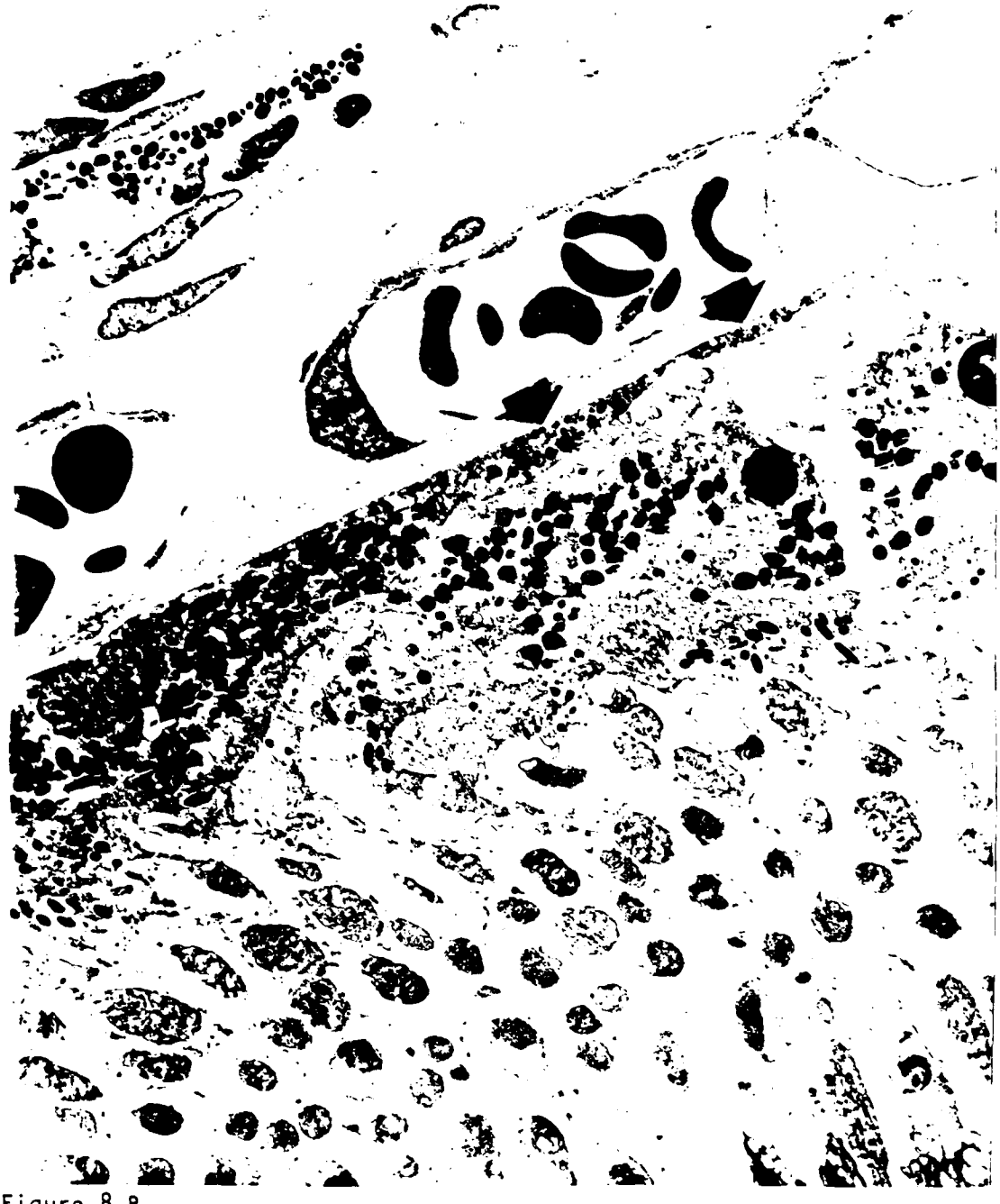


Figure 8 B

One edge of the lesion showing cytoplasmic extension (arrow) of marginal retinal pigment epithelial cell. x 2500

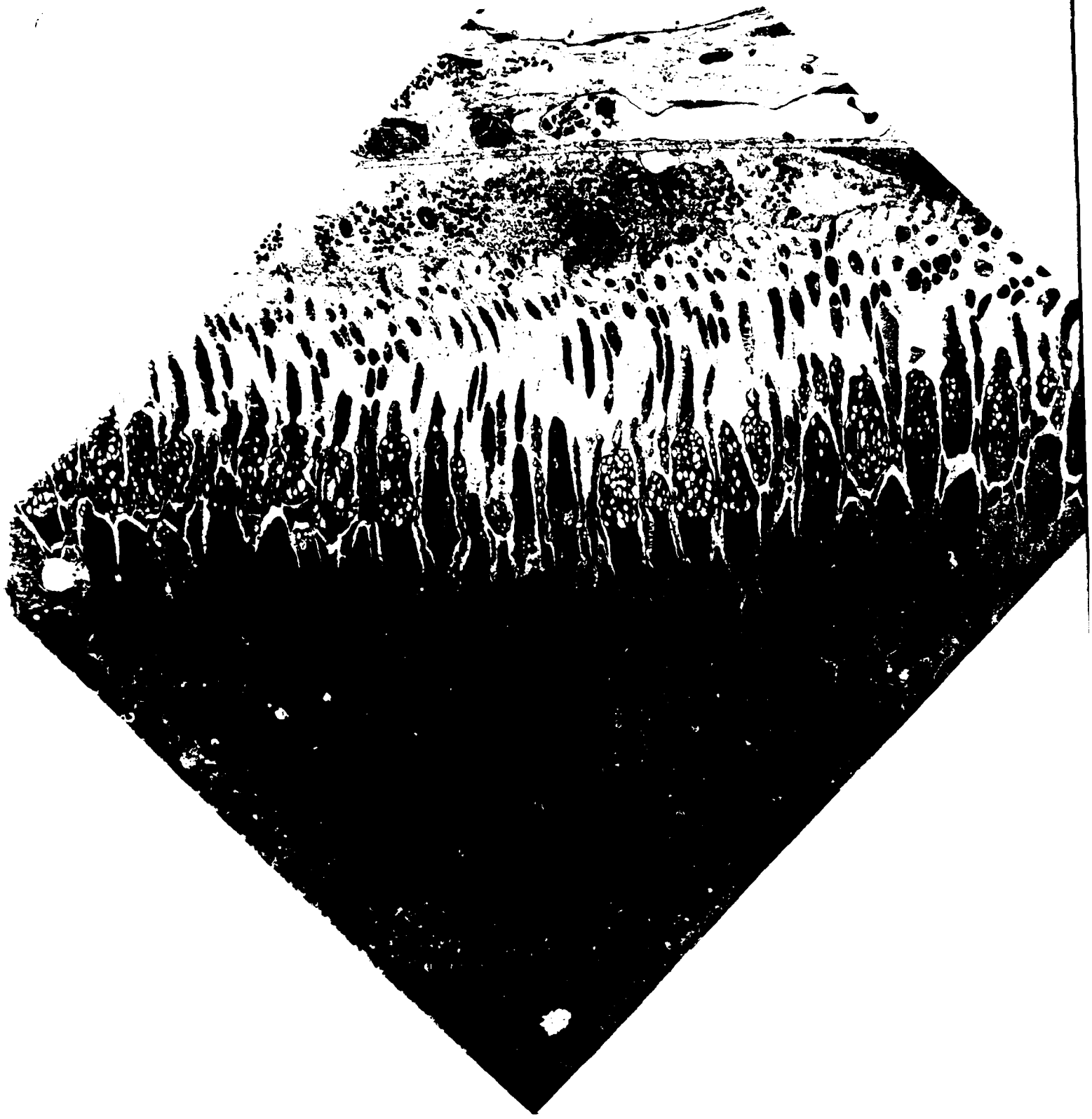


Figure 9 - Right Eye: One hour marker burn

In addition to disruption of retinal pigment epithelium there is destruction of the outer and inner segments of rods and cones. Cellular debris is dispersed over the extent of the lesion. The affected rod and cone nuclei appear pyknotic. x 1000

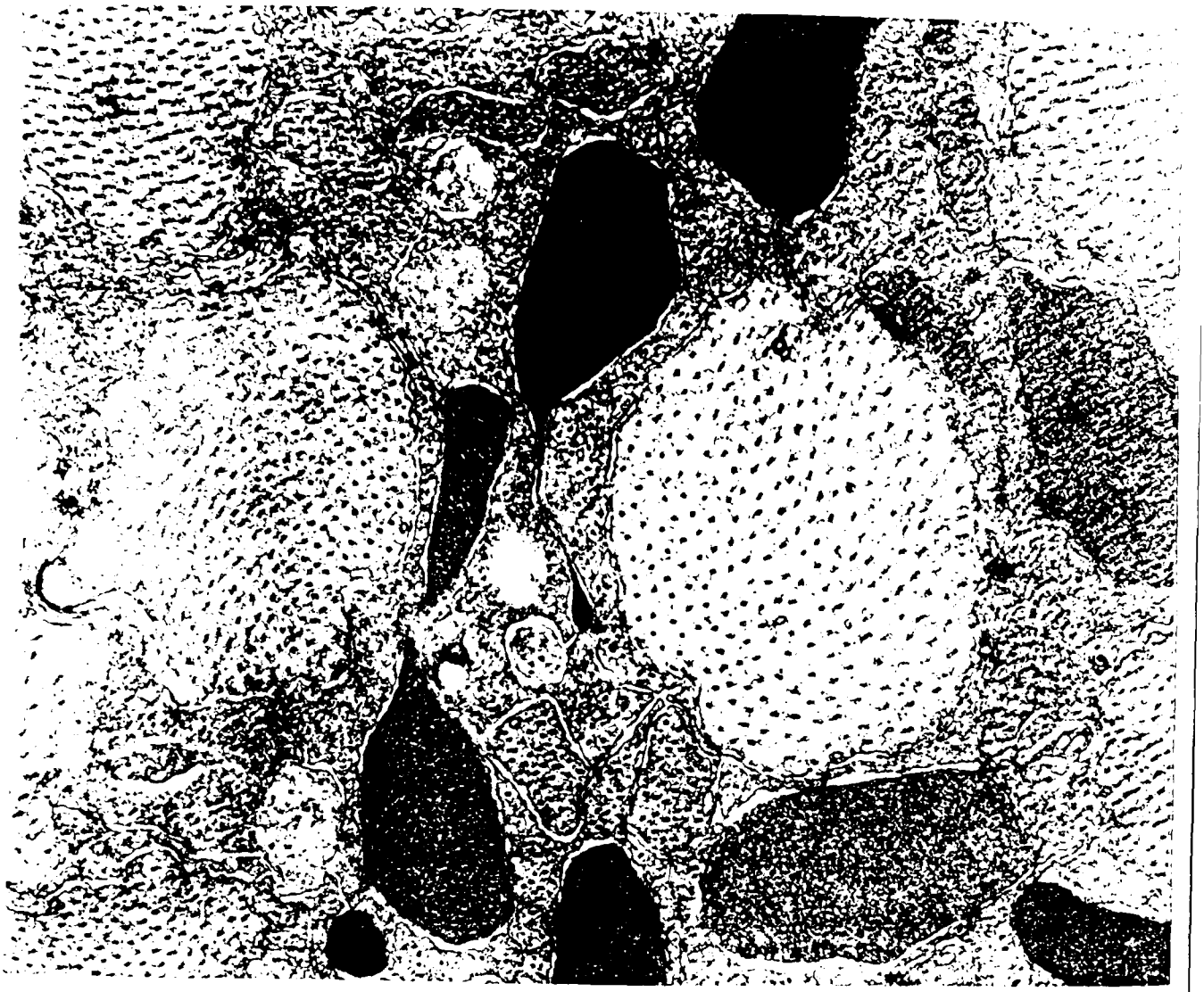


Figure 10 - Right Eye: One hour marker burn

Dark staining material is visible in cross sections of axons in the outer plexiform layer. x 16,500

Figure 11 A - Right Eye: One hour lesions

Two one hour lesions adjacent to each other. The retinal pigment epithelium within the confines of the lesion is separated from Bruchs' membrane and most of these cells exhibit severe damage. The undamaged retinal pigment epithelial cells at the margin of the lesion have developed tapered cellular extensions. x 560

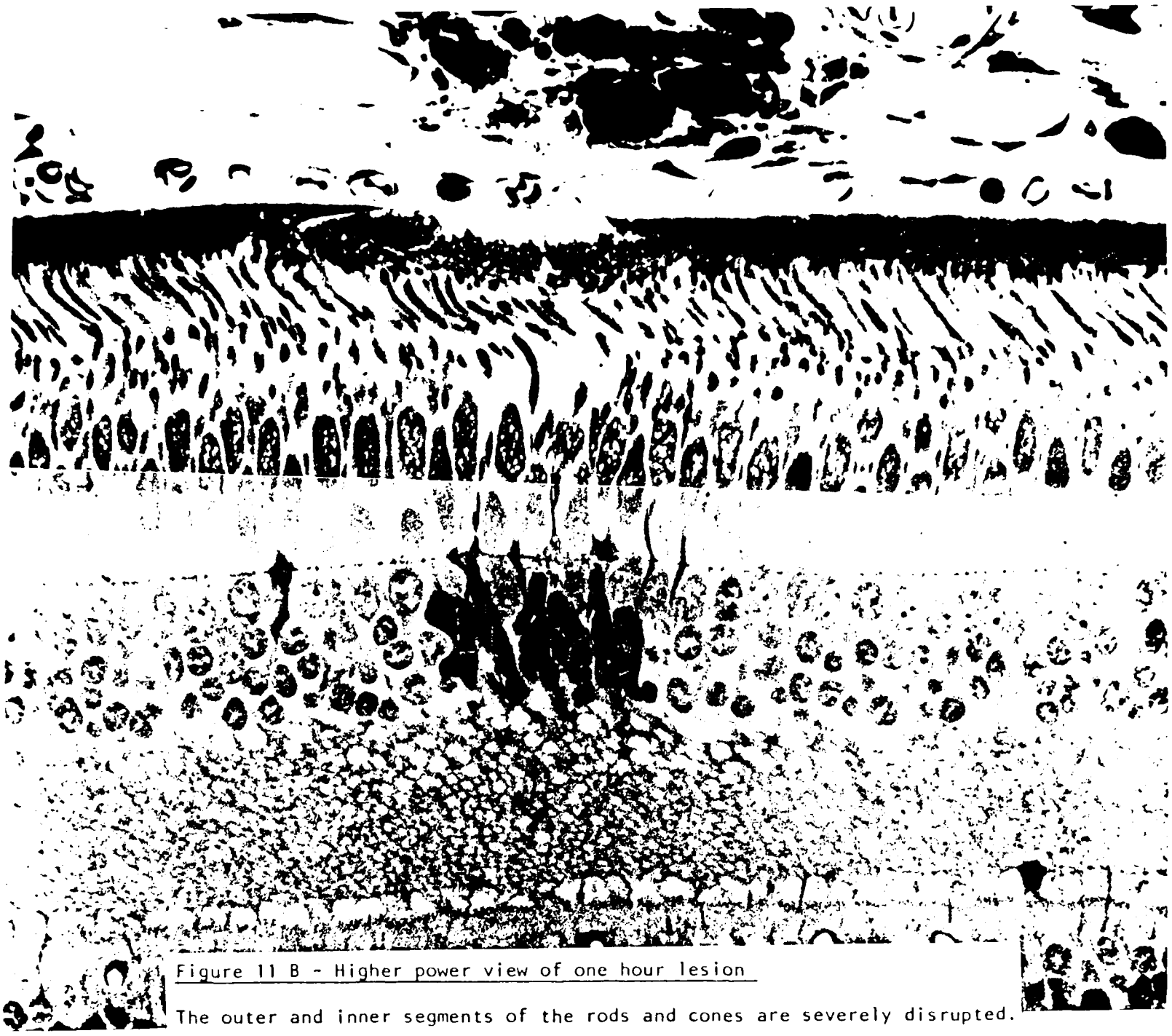


Figure 11 B - Higher power view of one hour lesion

The outer and inner segments of the rods and cones are severely disrupted. The affected nuclei exhibit pyknosis and appear deeply stained. x 900

Figure 12 - Right Eye: One hour lesion

Dark staining axons are identified in the outer plexiform layer and appear to be emanating from the pyknotic rod and cone nuclei (arrows). The axons are cut in longitudinal section and appear here as streaks. Compare to their appearance in figure 10 where they are seen in cross section. x 900

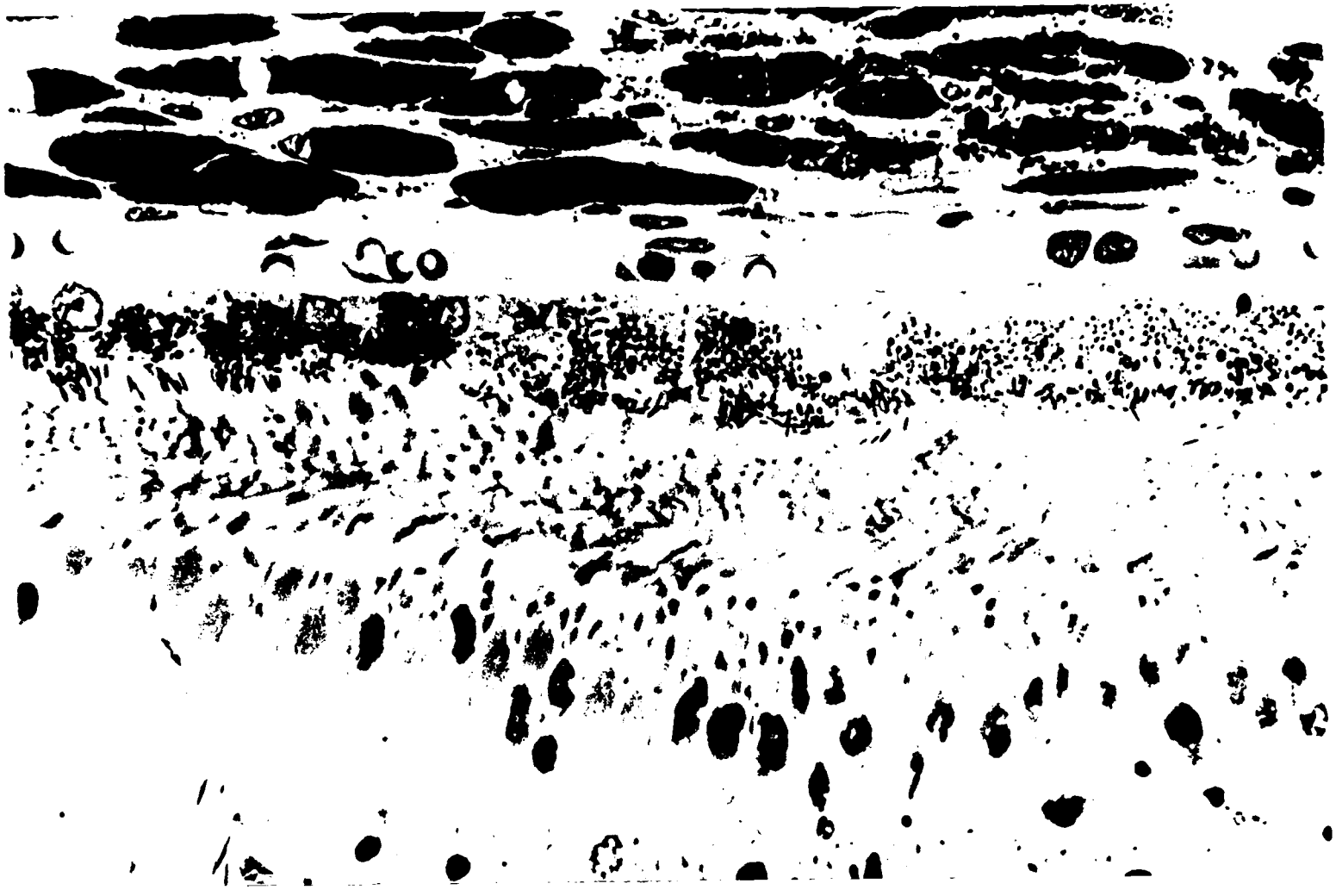


Figure 13 - Right Eye: One day marker burn

The choroid contains dispersed pigment granules and occasional free red

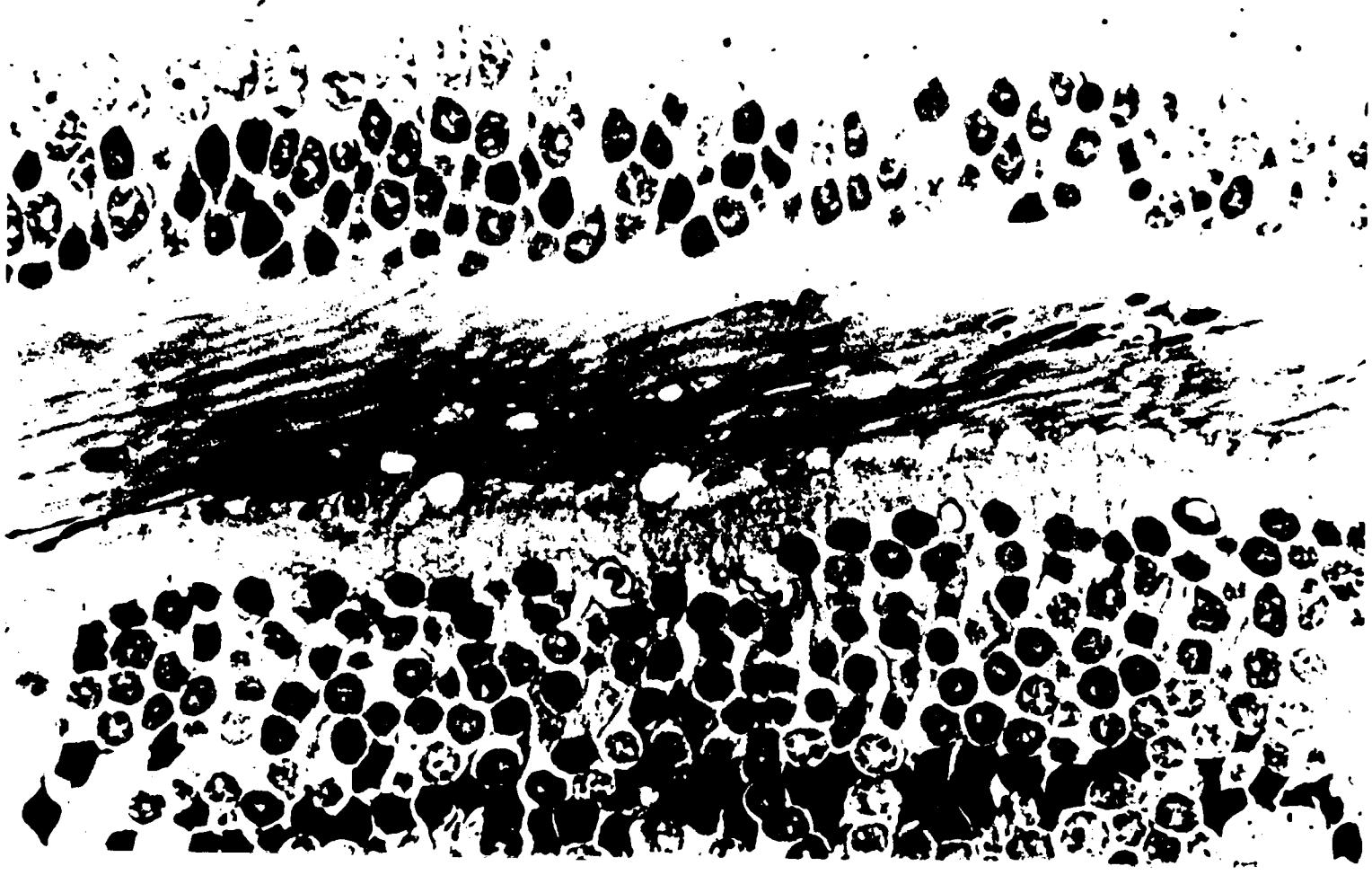


Figure 14 - Right Eye: One day marker burn

Dark staining axons are observed in the outer plexiform layer. Several are traceable to the area of pyknotic nuclei in the outer nuclear layer.

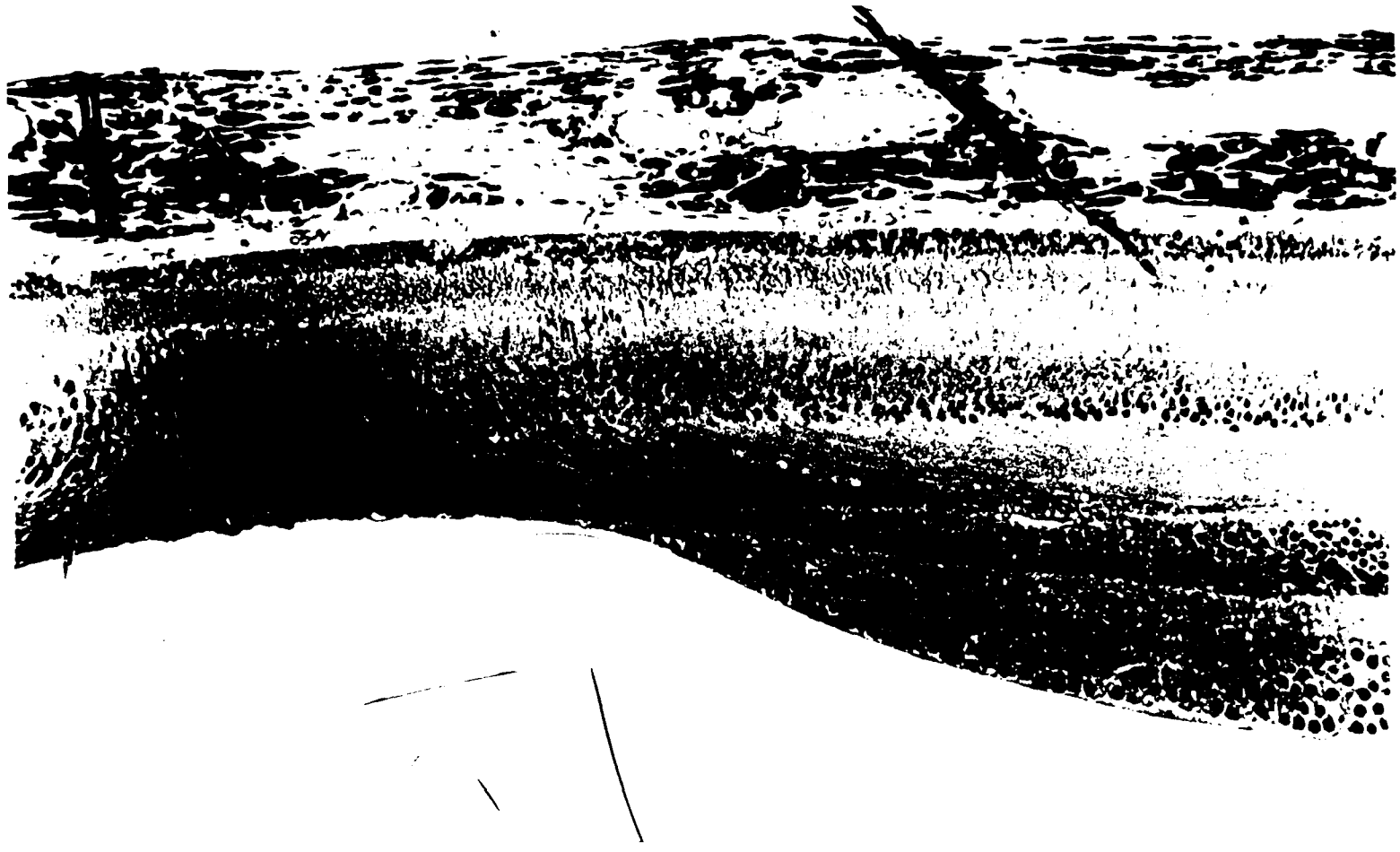


Figure 15 A - Right Eye: One day lesion

The lesion lies immediately adjacent to the foveola. The retinal pigment epithelium is disrupted with early extension of the cytoplasm of marginal cells into the lesion. In this lesion the retinal pigment epithelial cells

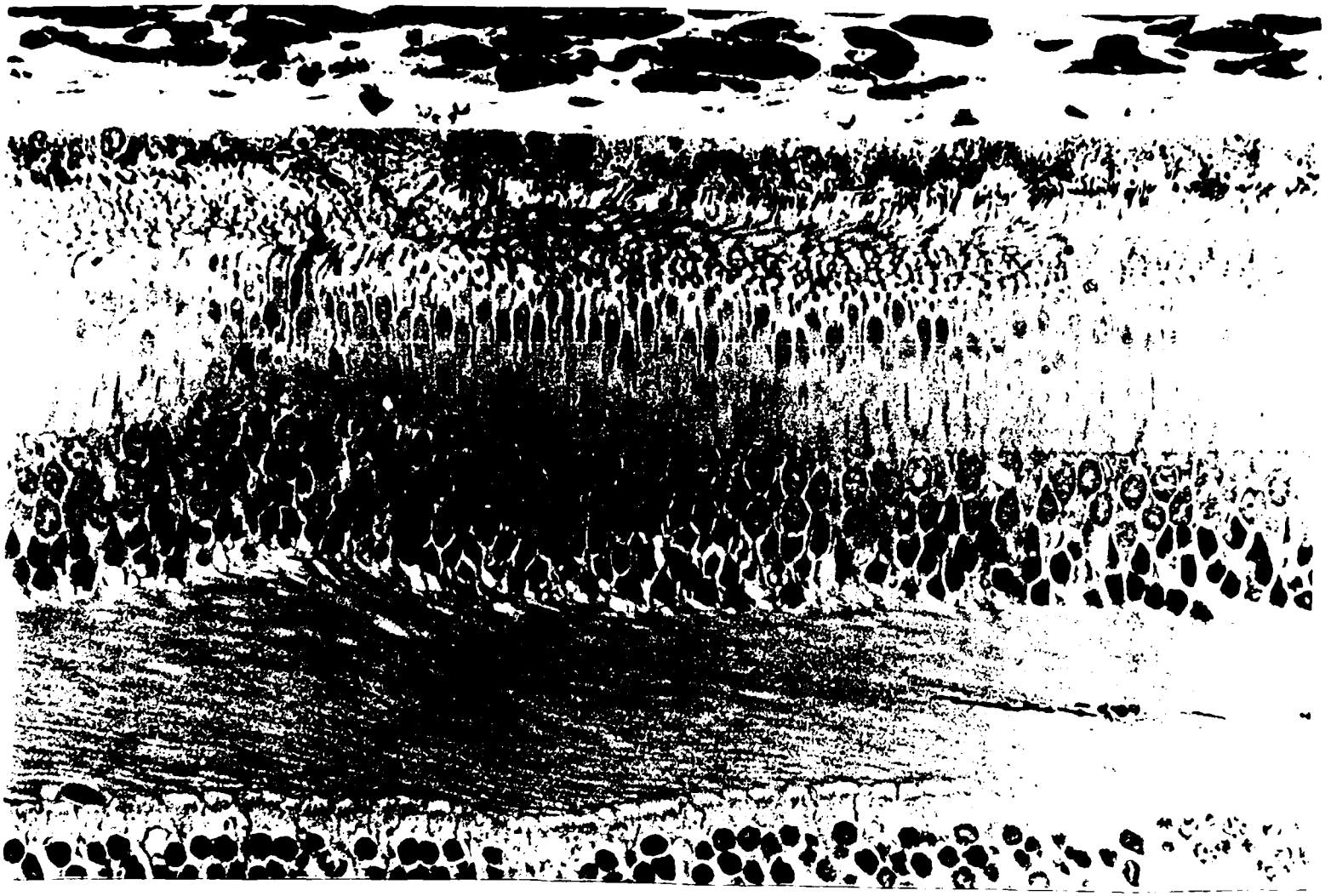


Figure 15 B - Right Eye:. One day lesion

At higher power pyknotic nuclei of affected rods and cones are seen.

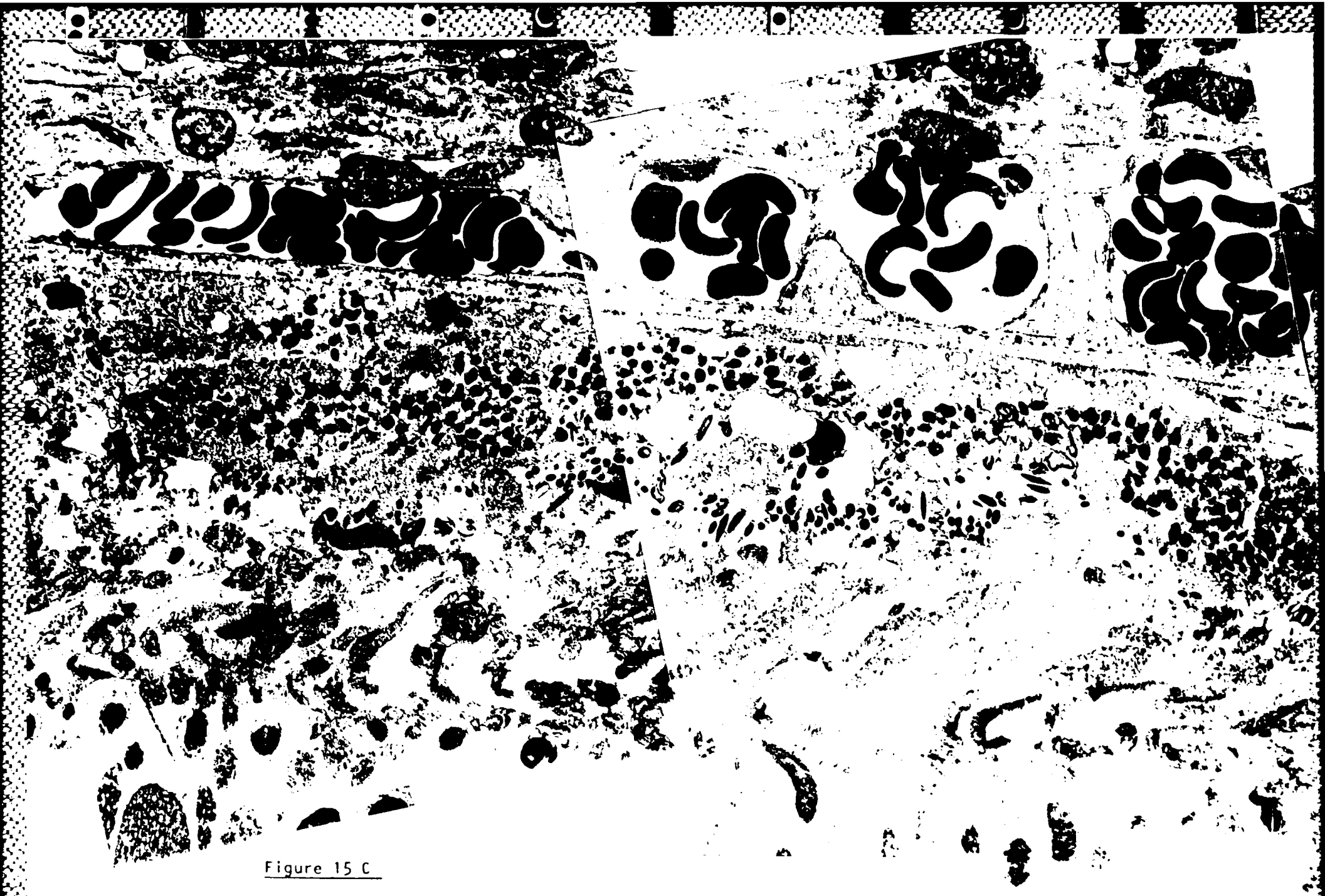


Figure 15 C

Tapered cytoplasmic processes from the marginal retinal pigment epithelial cells extend into the site of injury (arrows). The damaged retinal pigment epithelial cells and disrupted outer segments of the rods and cones are quite evident. x 2500

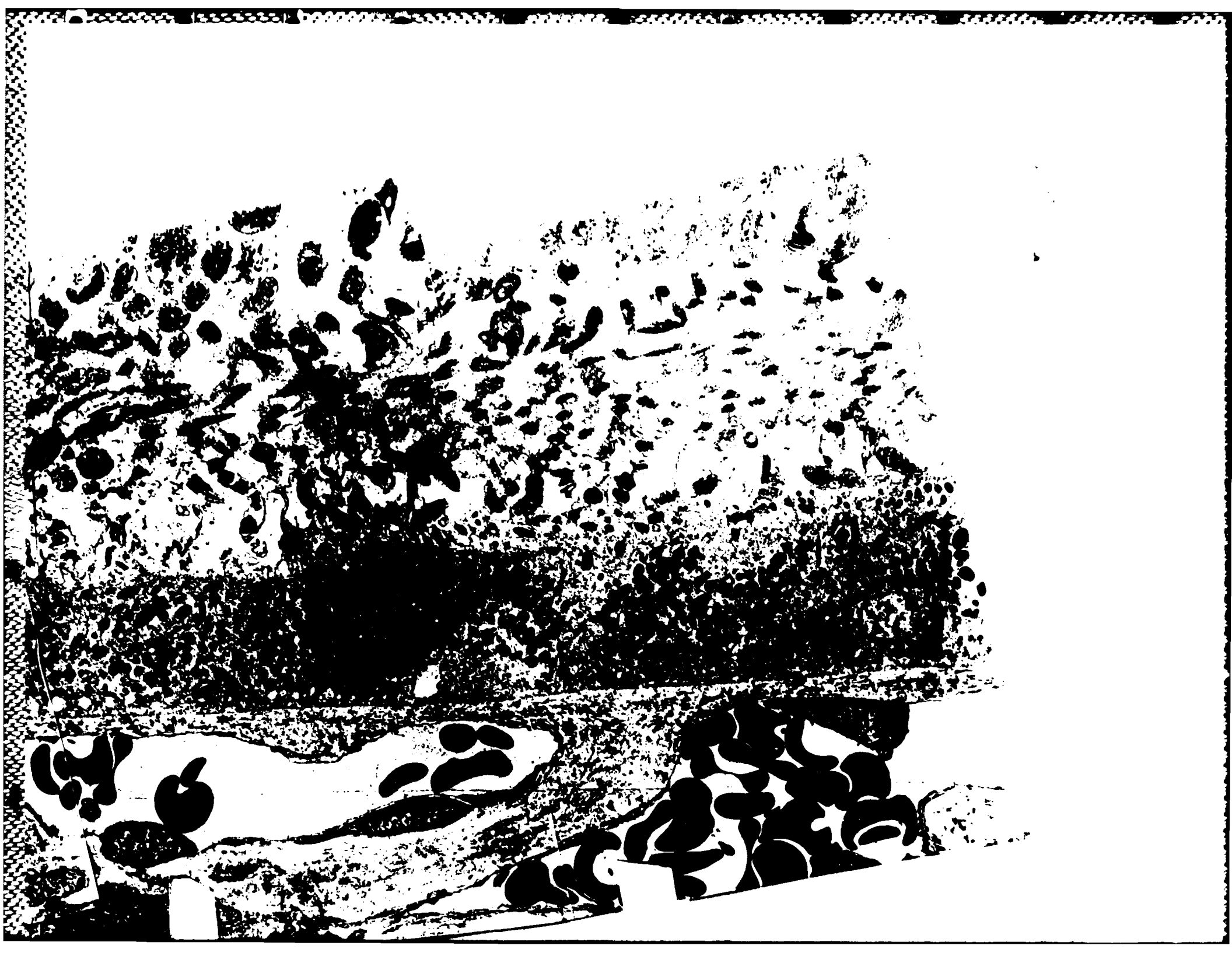


Figure 16 - Right Eye: One week marker burn

Macrophages are seen in the choroid (black arrow) and in the retina (white arrow). Note football shaped granules characteristic of retinal pigment epithelium in both areas. x 7500

Figure 17 A - Right Eye: One week marker burns

Fortuitous s ctions show a pigment-containing cell that has partially passed between the retina and the choroid through Bruchs' membrane. Similar cells presumed to be macrophages are observed on the retinal side of Bruchs' membrane. The outer segment rod and cone debris has largely disappeared. x 560

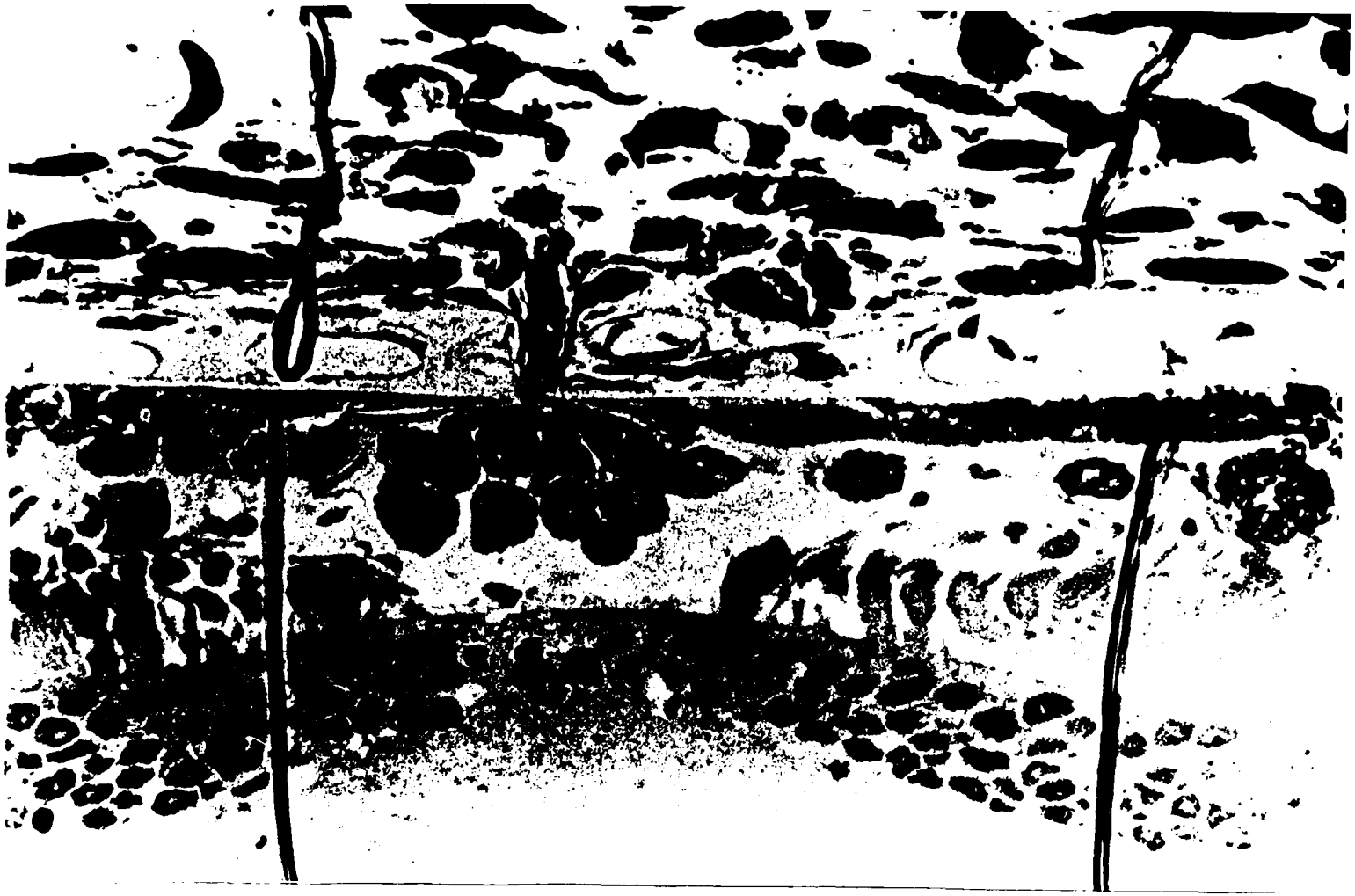


Figure 17 B - Right Eye: One week marker burn

Higher magnification of 17 A showing football shaped retinal pigment epithelial granules within the cells believed to be macrophages. x 900

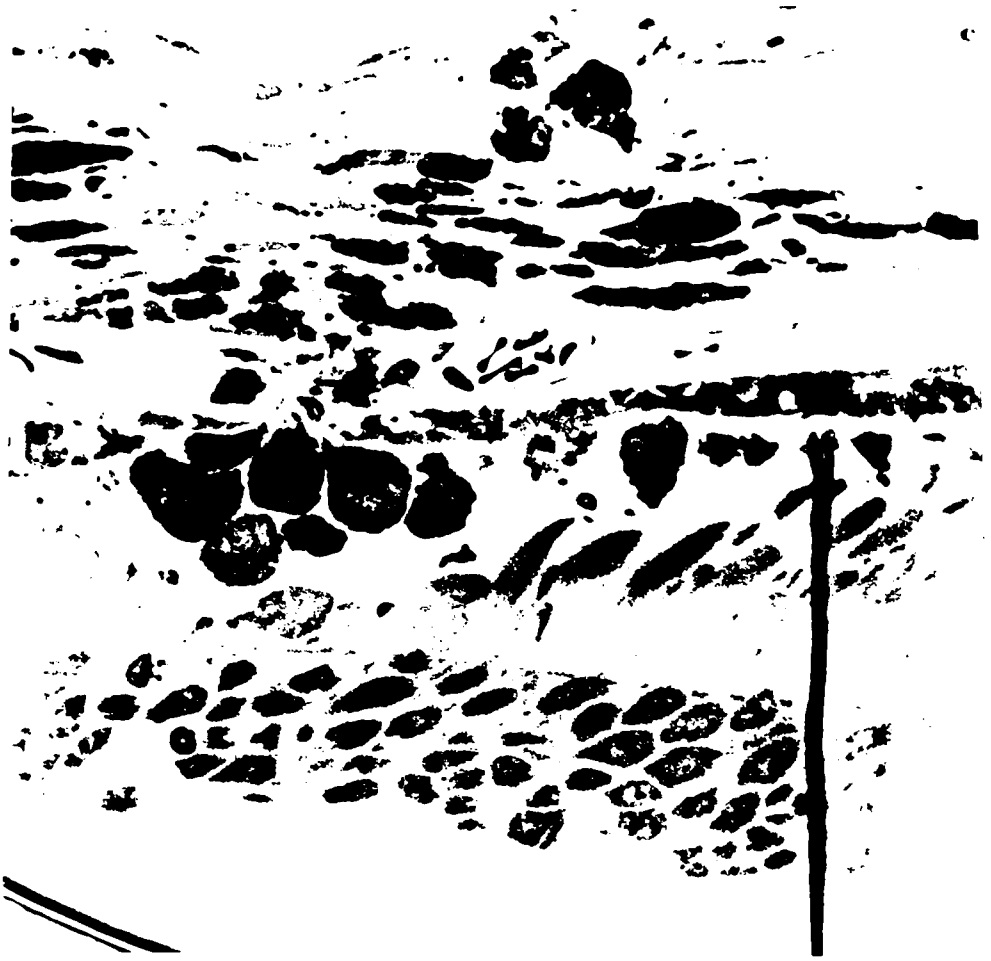


Figure 18 - Right Eye: One week marker burn

Another fortuitous section from another burn showing the same types of cells as observed in figures 17 A and B passing between the retina and the choroid through Bruchs' membrane. x 900

Figure 19 A - Right Eye: One week marker burn

A few pyknotic nuclei are seen in the outer nuclear layer. The residual cells in the outer nuclear layer are few in number than the surrounding normal retina. x 560

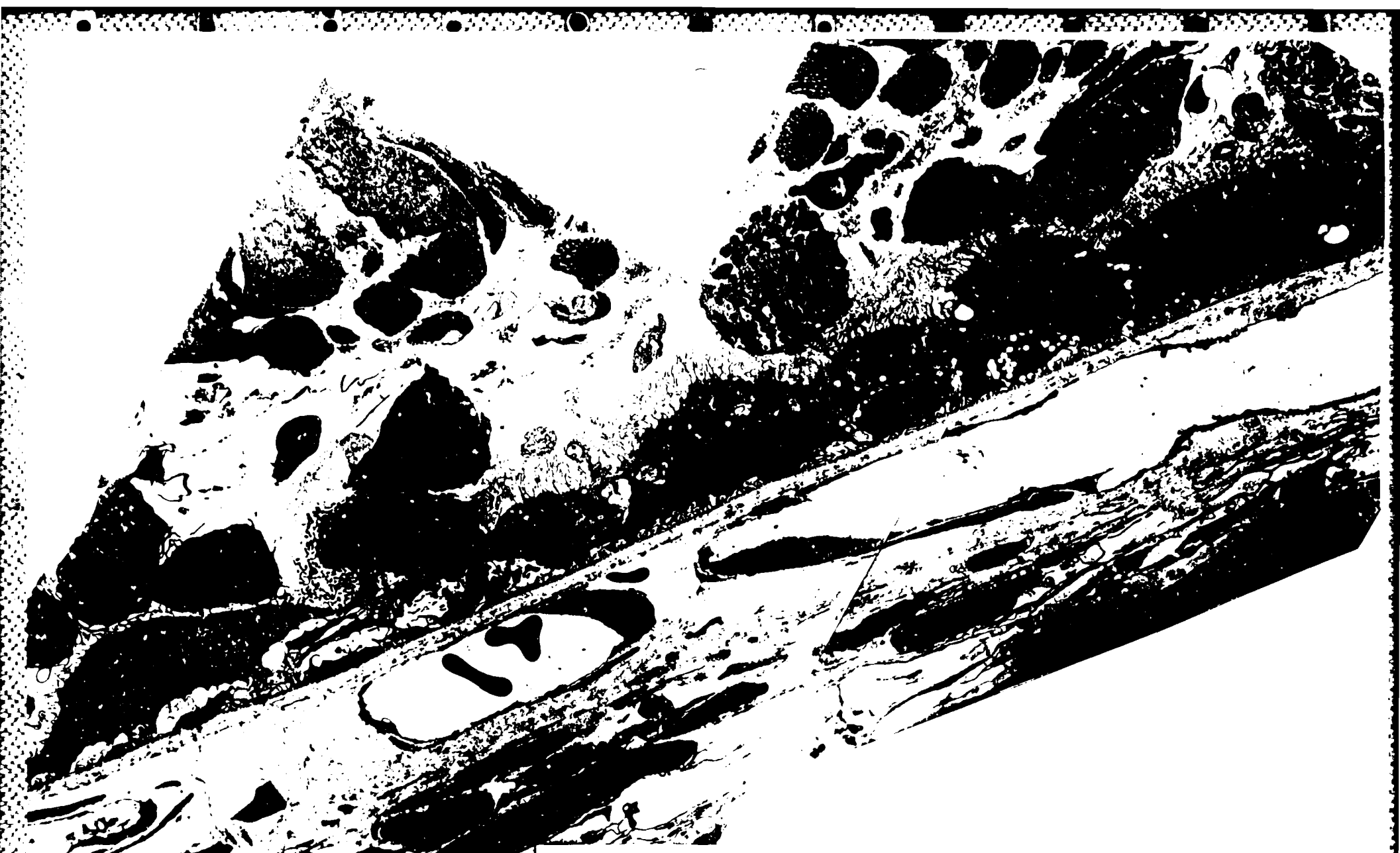


Figure 19 B - Right Eye

At higher magnification the retinal pigment epithelial cells that have migrated into the lesion site now cover Bruchs' membrane. They possess fewer pigment granules and are thinner than normal retinal pigment epithelial cells. They exhibit interdigitating cellular processes and complex basilar and apical infoldings and microvilli. Numerous macrophages containing engulfed pigment granules are also seen. x 2500

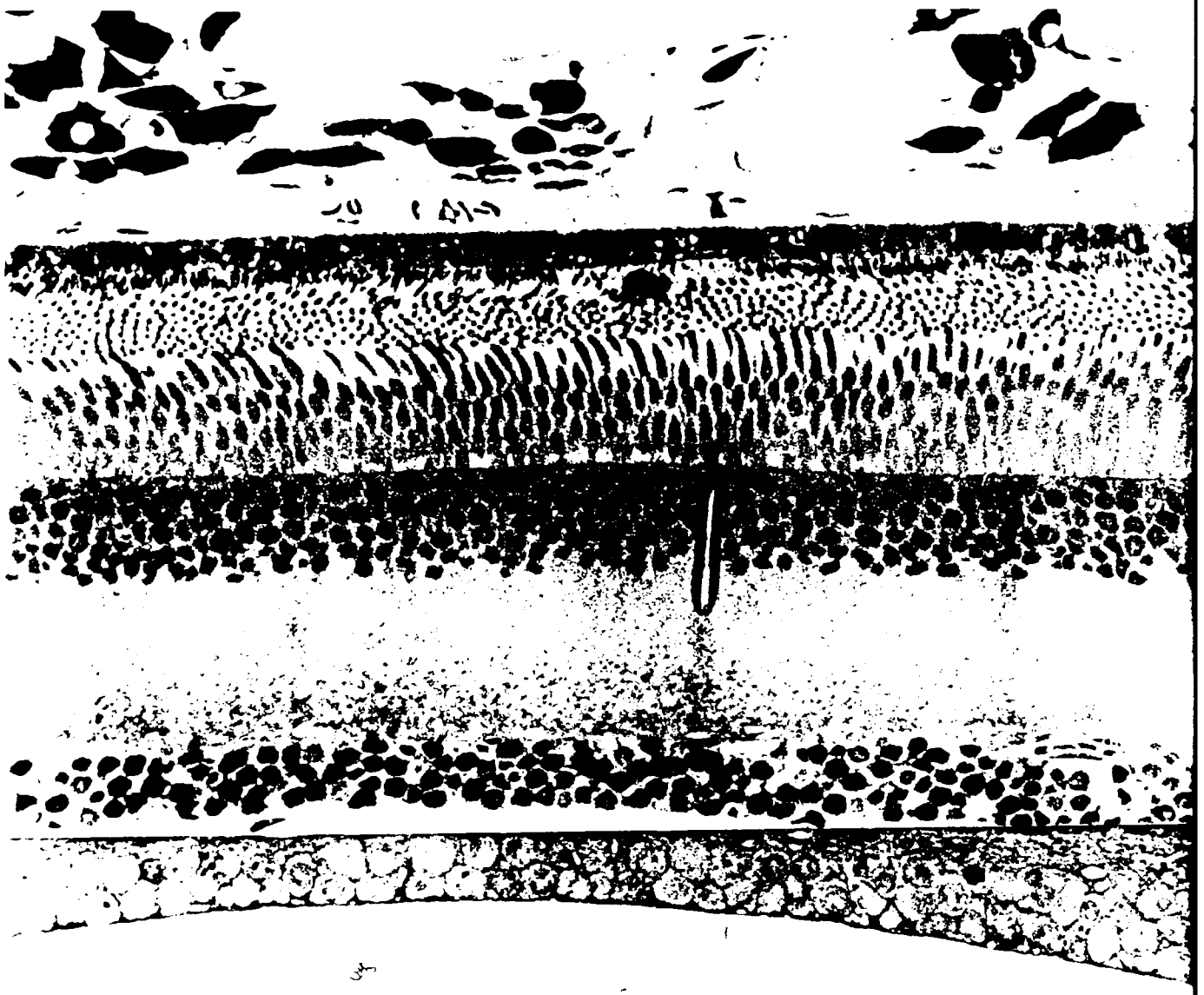
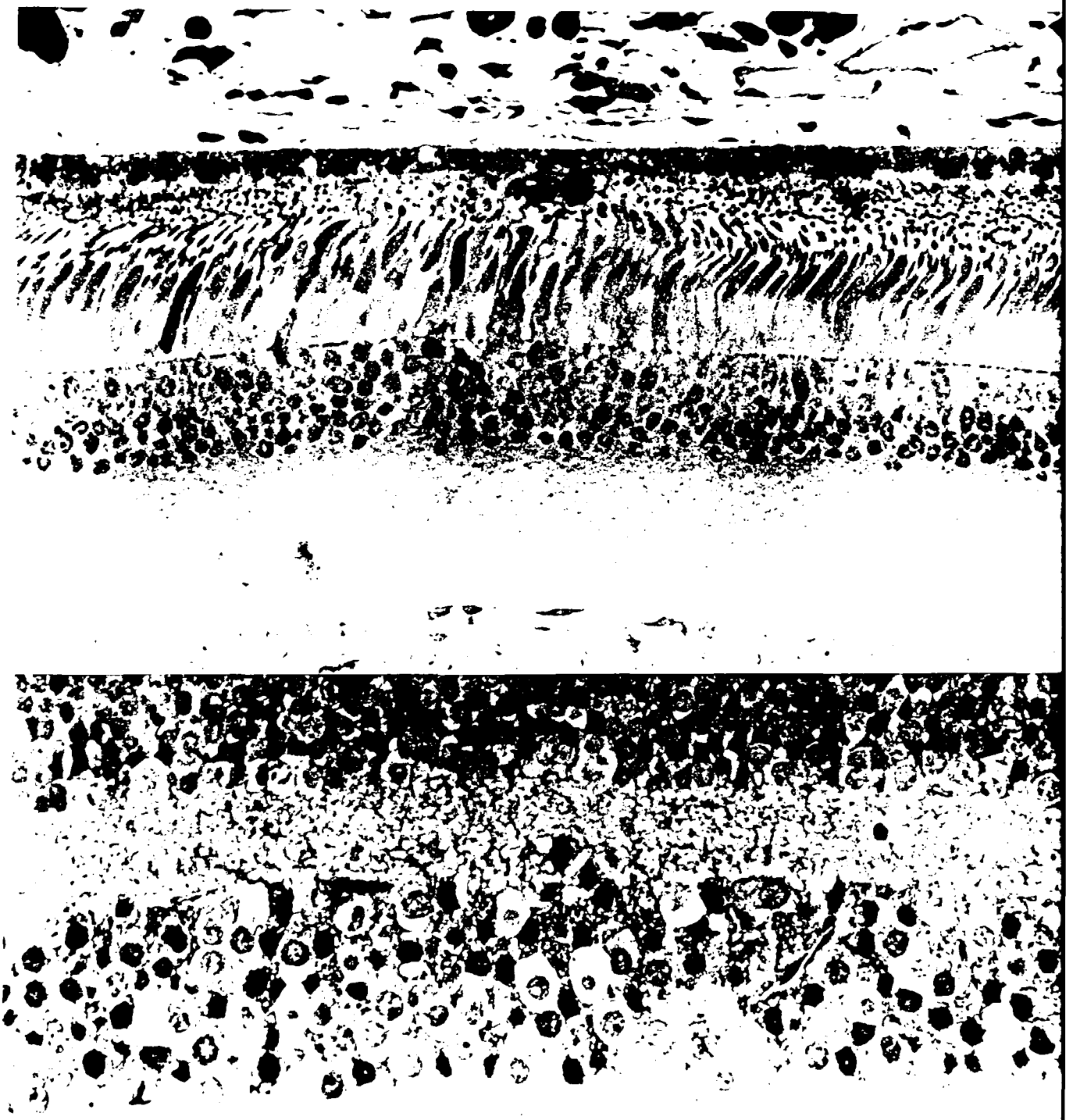


Figure 20 - Right Eye. One week lesion

The retinal pigment epithelium has almost completely covered the defect. Debris and macrophages are present. Much of the debris has been cleared from the region of the RPE and some outer segment destruction. x 560



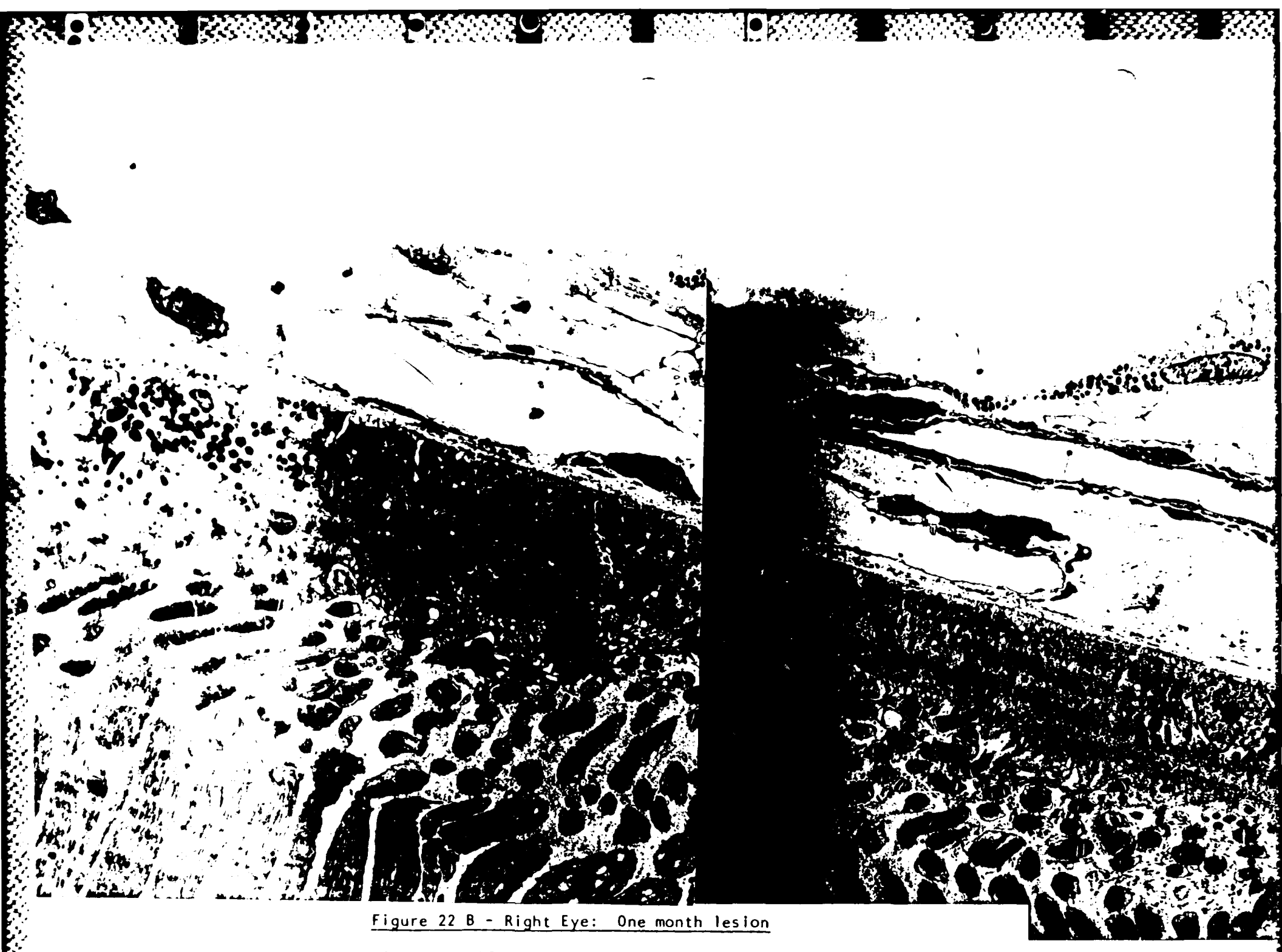


Figure 22 B - Right Eye: One month lesion

Higher magnification to show paucity of pigment in reparative retinal

Figure 23 - Left Eye: One hour marker burn

Round cells resembling lymphocytes (arrows) are seen within the choriocapillaris and deeper choroidal vessels adjacent to the lesion. x 560

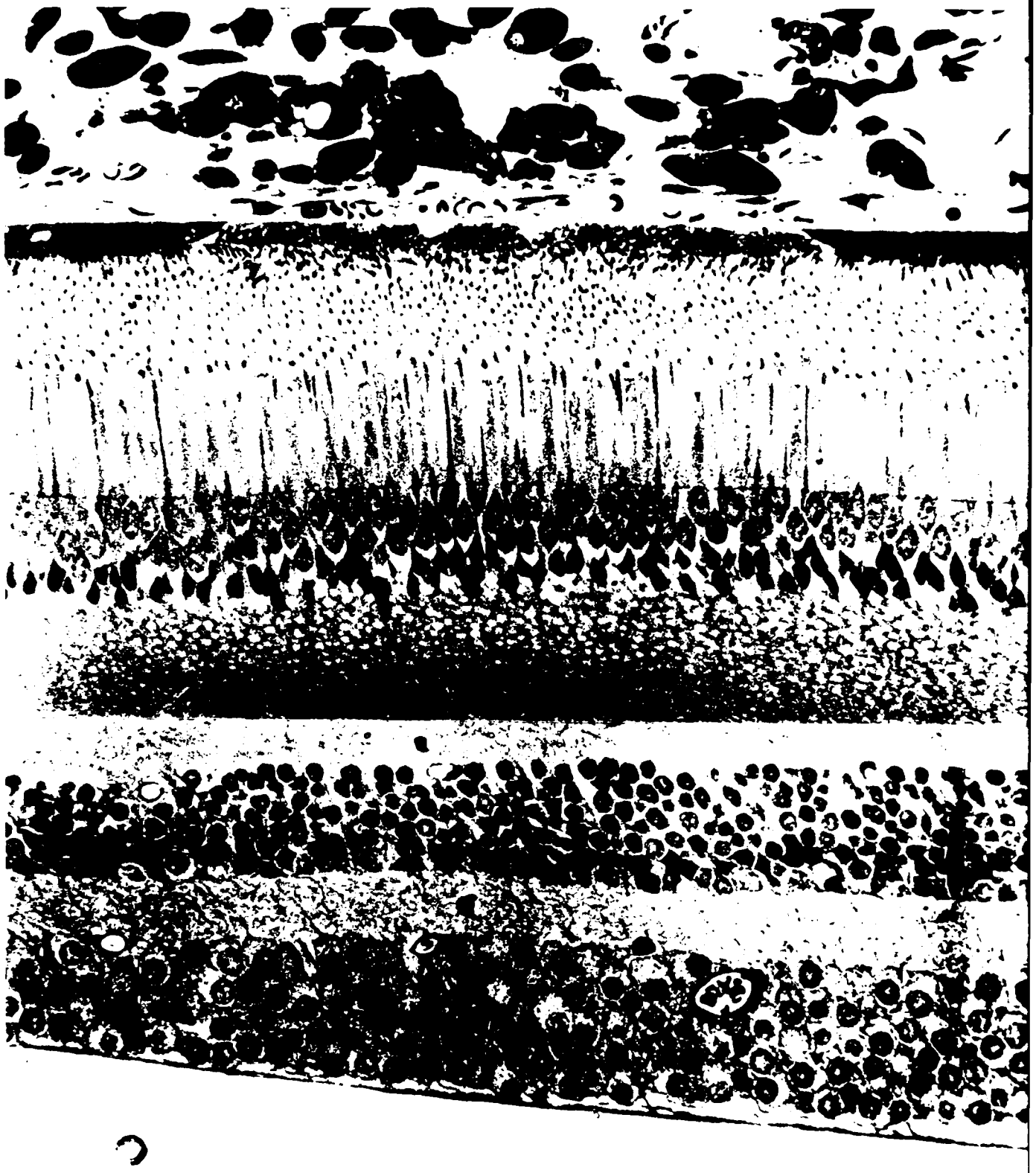
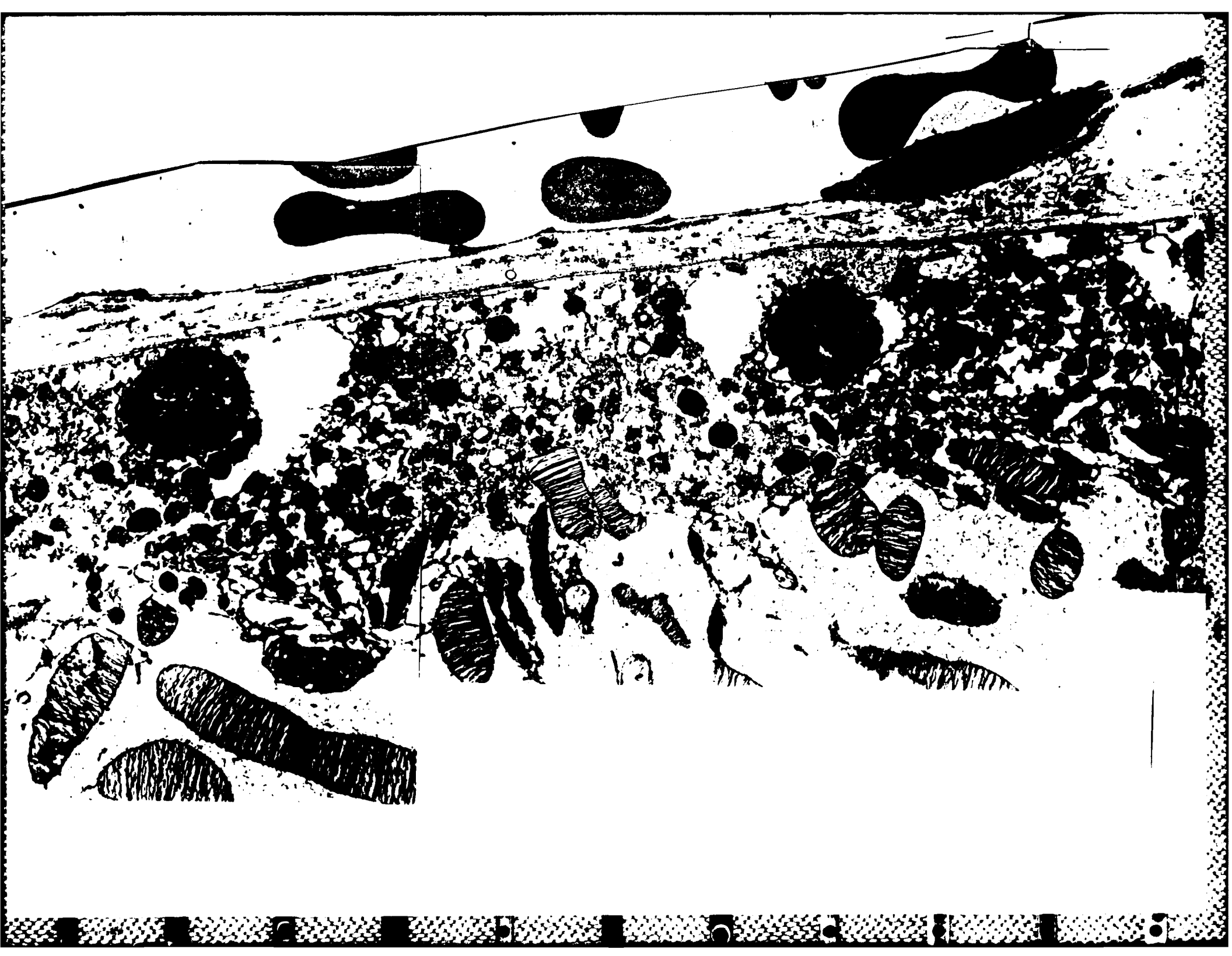


Figure 24 - Left Eye: One hour marker burn

The retinal pigment epithelium is disrupted within the confines of the lesion but it is not displaced away from Bruchs' membrane. The pigment epithelial cells at the edge of the lesion exhibit cytoplasmic extensions toward its center. No macrophages are seen. x 560

Figure 25 - Left Eye: One hour marker burn

The outer segments of the rods and cones are partially disrupted but several



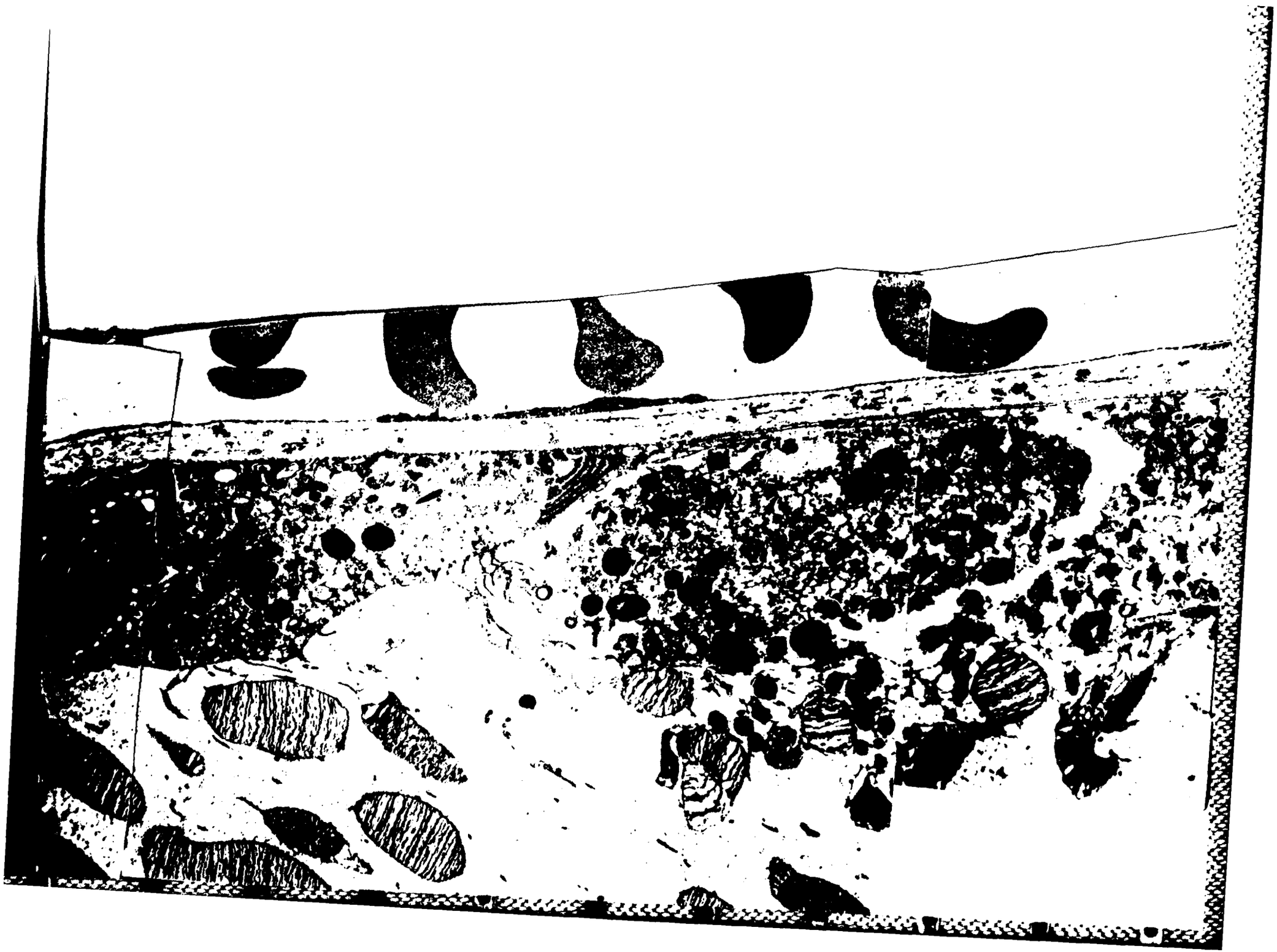
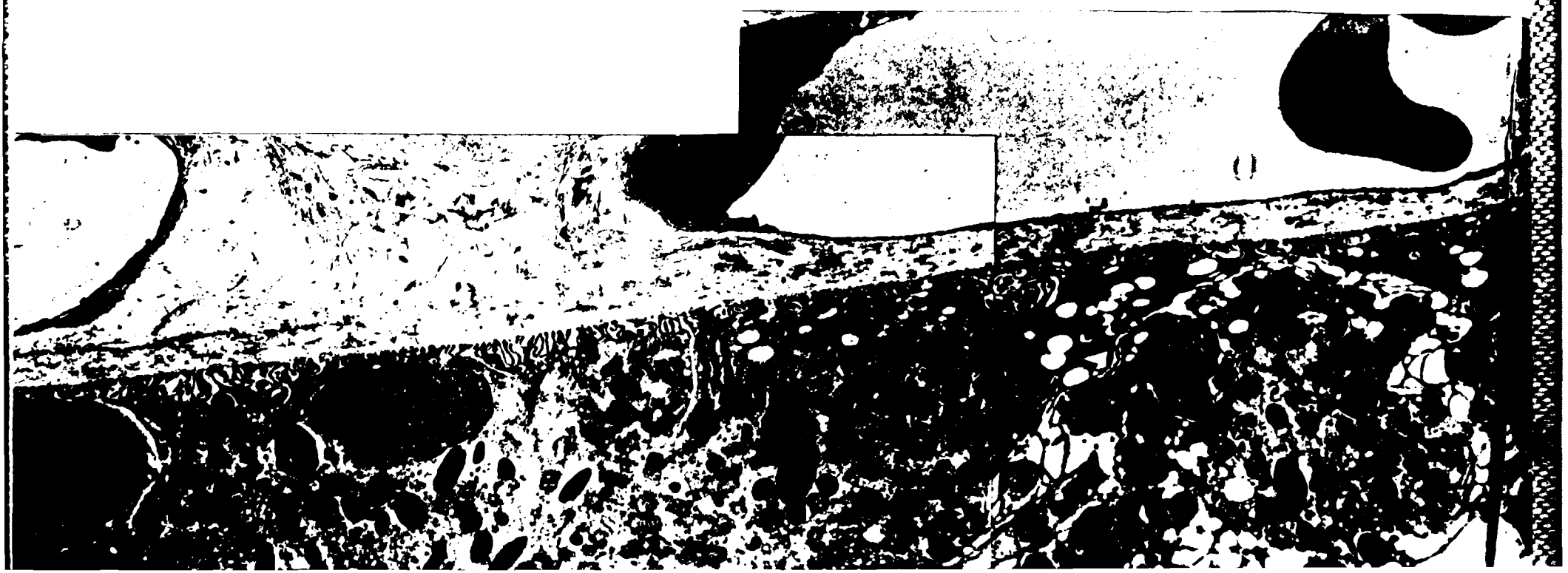
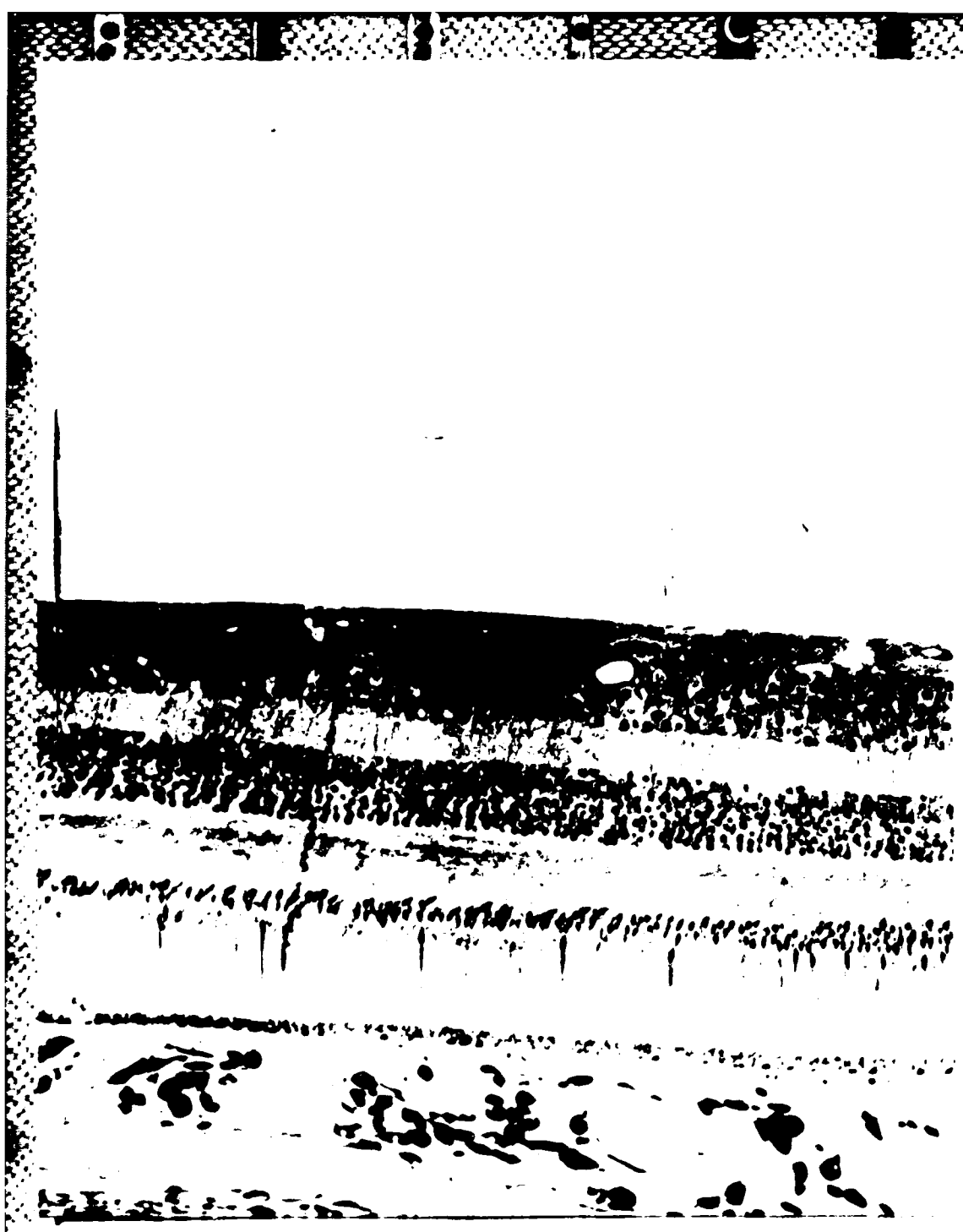


Figure 26 - Left Eye: Higher magnification of one hour marker burn

This shows cytoplasmic extension of RPE cells toward the center of the lesion. Disrupted RPE cells are noted in the center of the lesion. Note relatively well preserved outer segment material. x 7500





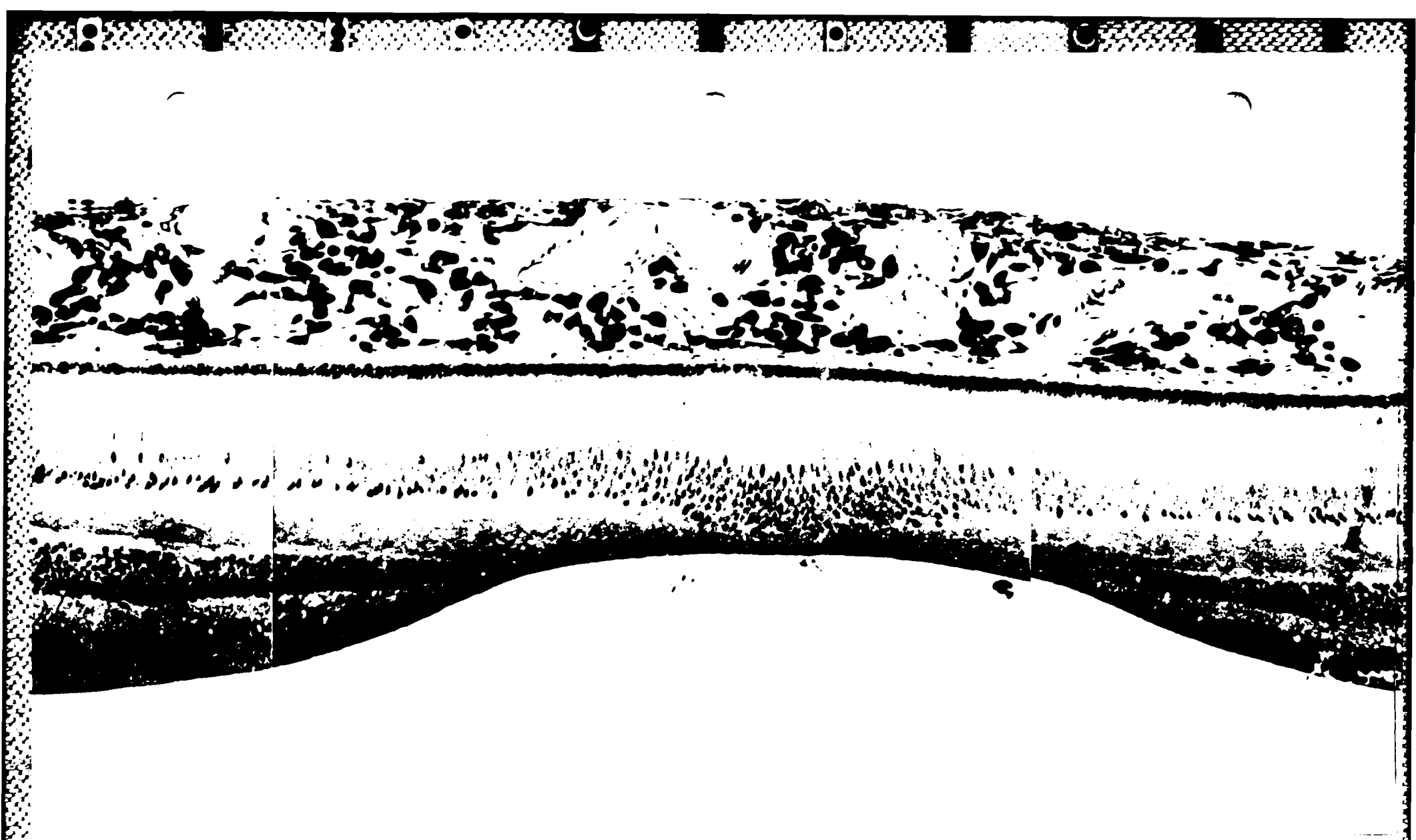


Figure 27 A - Left Eye : One hour lesion

A miniscule lesion is observed almost in the center of the foveola. The choroid appears unaffected without evidence of choroidal hemorrhage or dispersed pigment. × 225

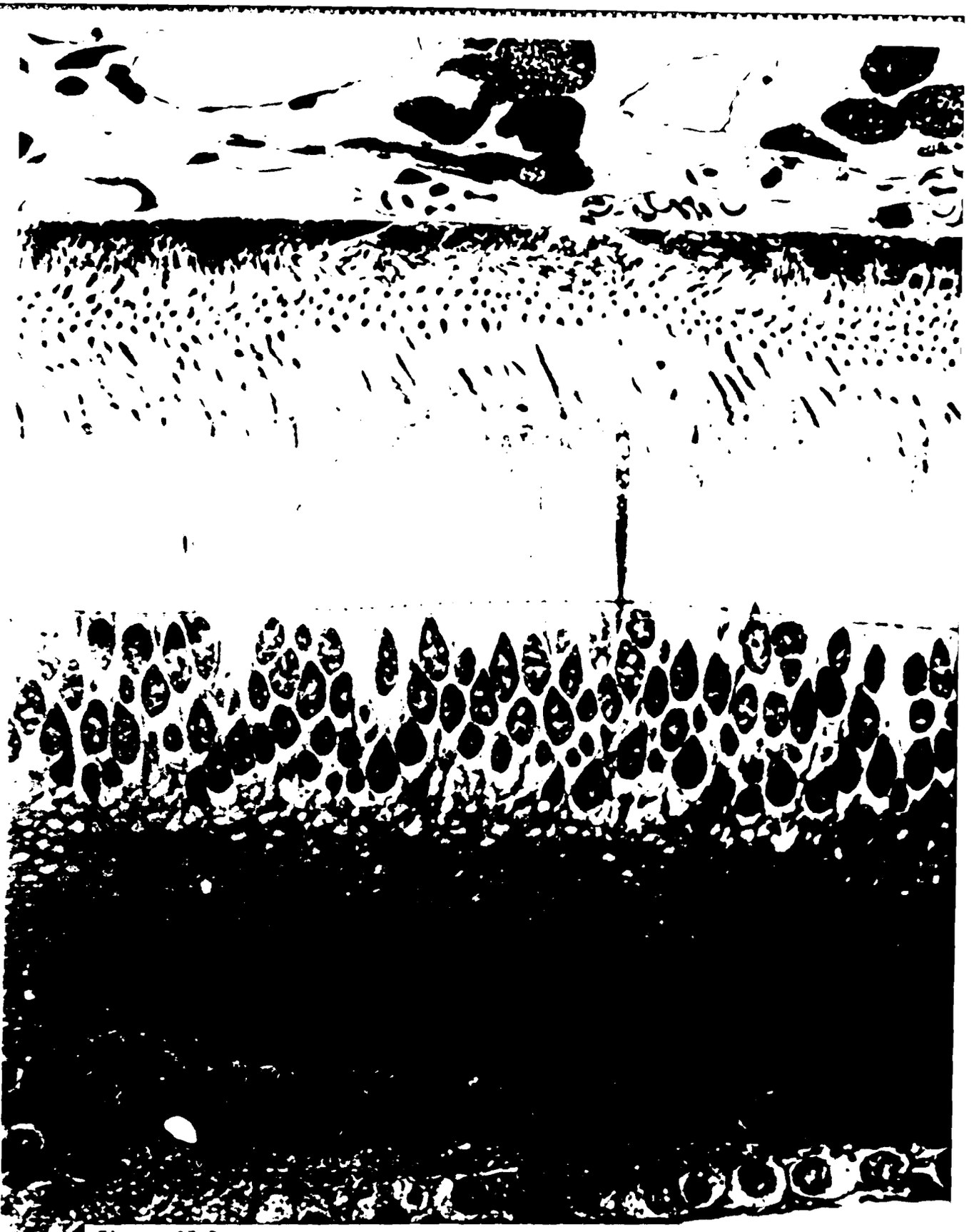


Figure 27 B

Undamaged RPE cells at the margins of the lesion exhibit extensions of the cytoplasm toward the area of retinal pigment epithelial sloughing. The dark staining cells in the outer portion of the outer nuclear layer are normal and are seen throughout the retina as well as in the area of the lesion. x 900

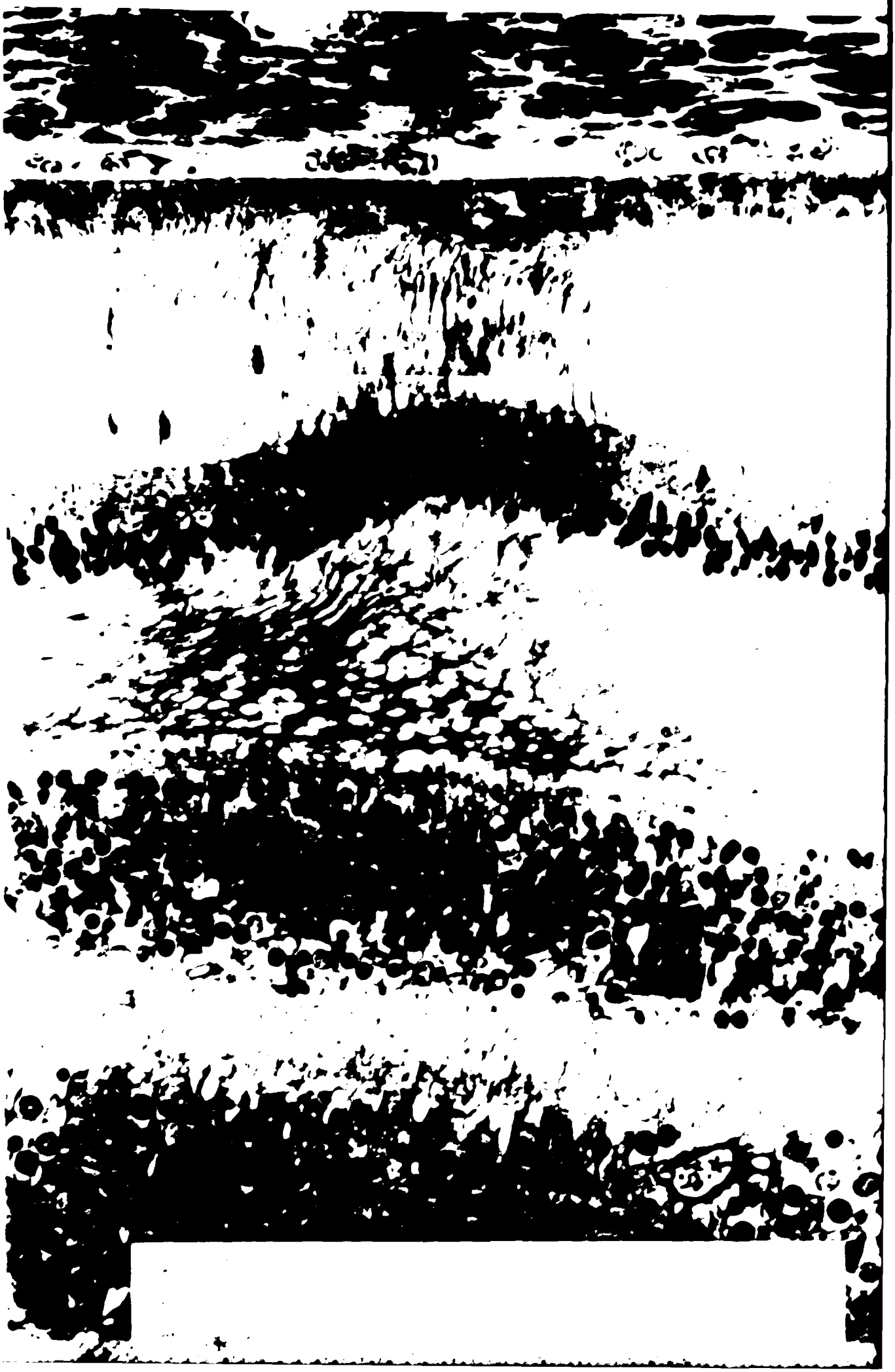
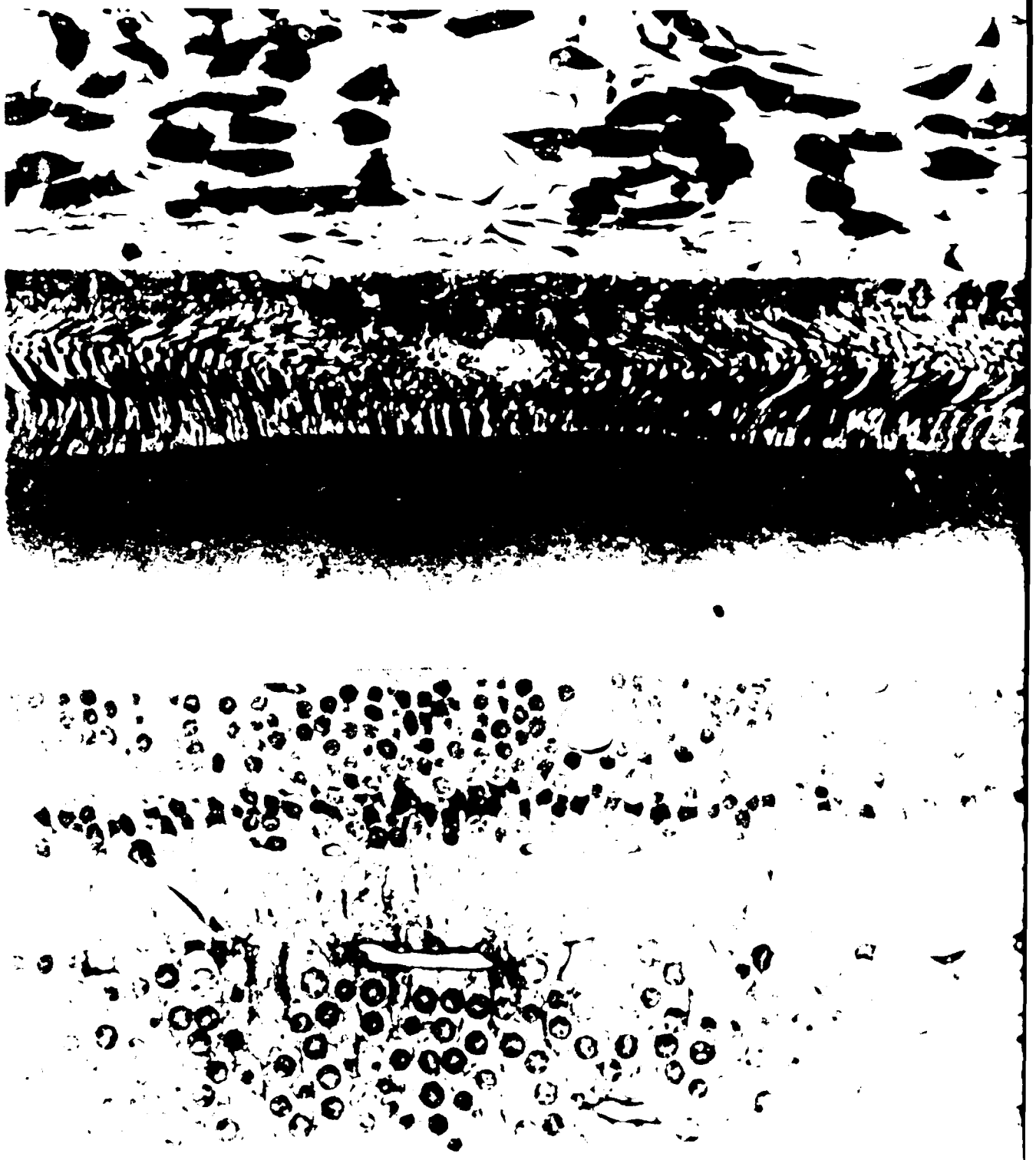


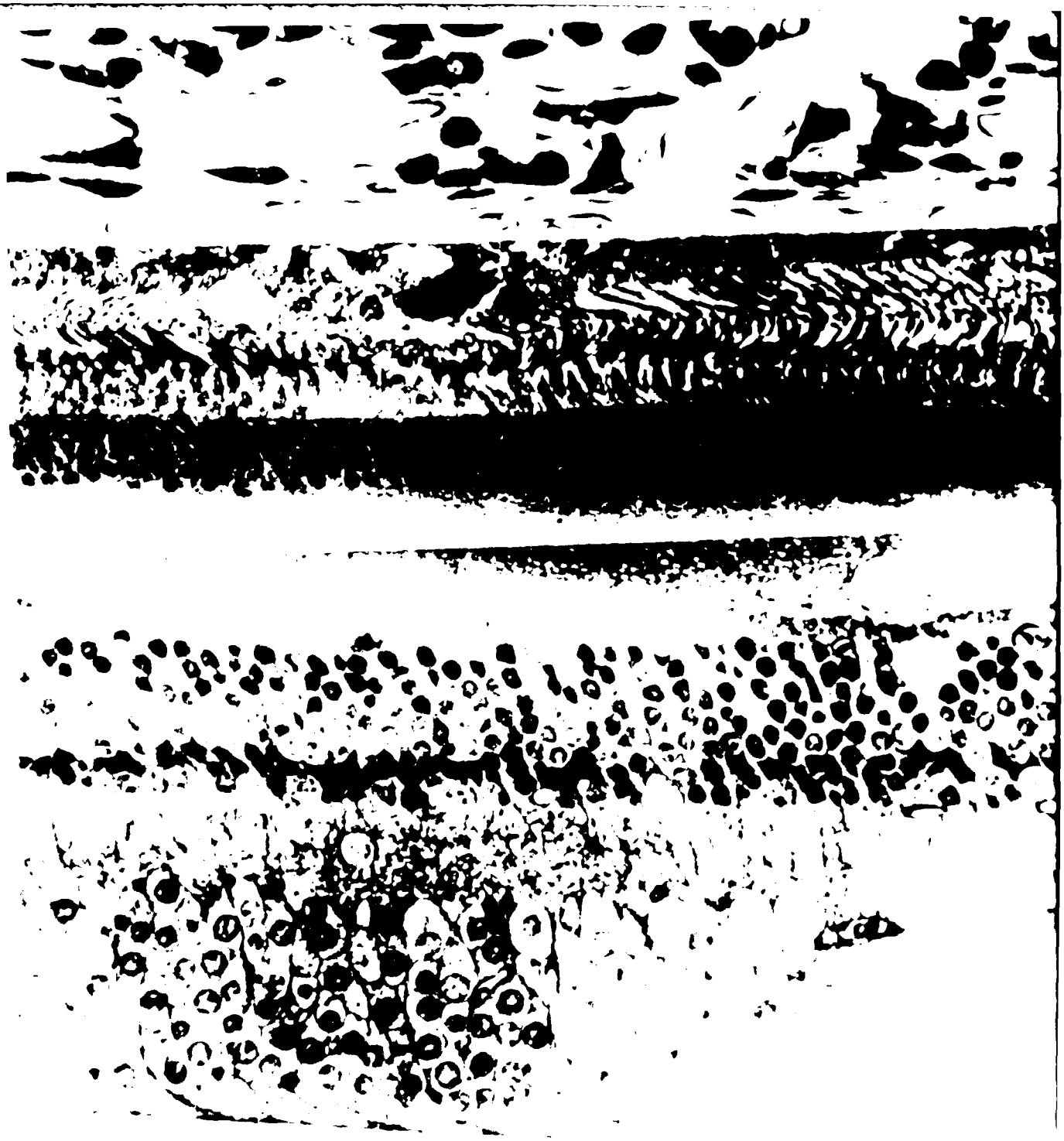
Figure 45 E - Left Eye - One day after light

Similar destruction of the outer limiting membrane is seen in this one hour after burn. Also note pyknosis of rod and cone inner segment material and dark staining axonal material in outer plexiform

Figure 29 - Left Eye: One day lesion

The damaged retinal pigment epithelium is separated from Bruchs' membrane. Several of the rod and cone inner segments appear pyknotic. x 560





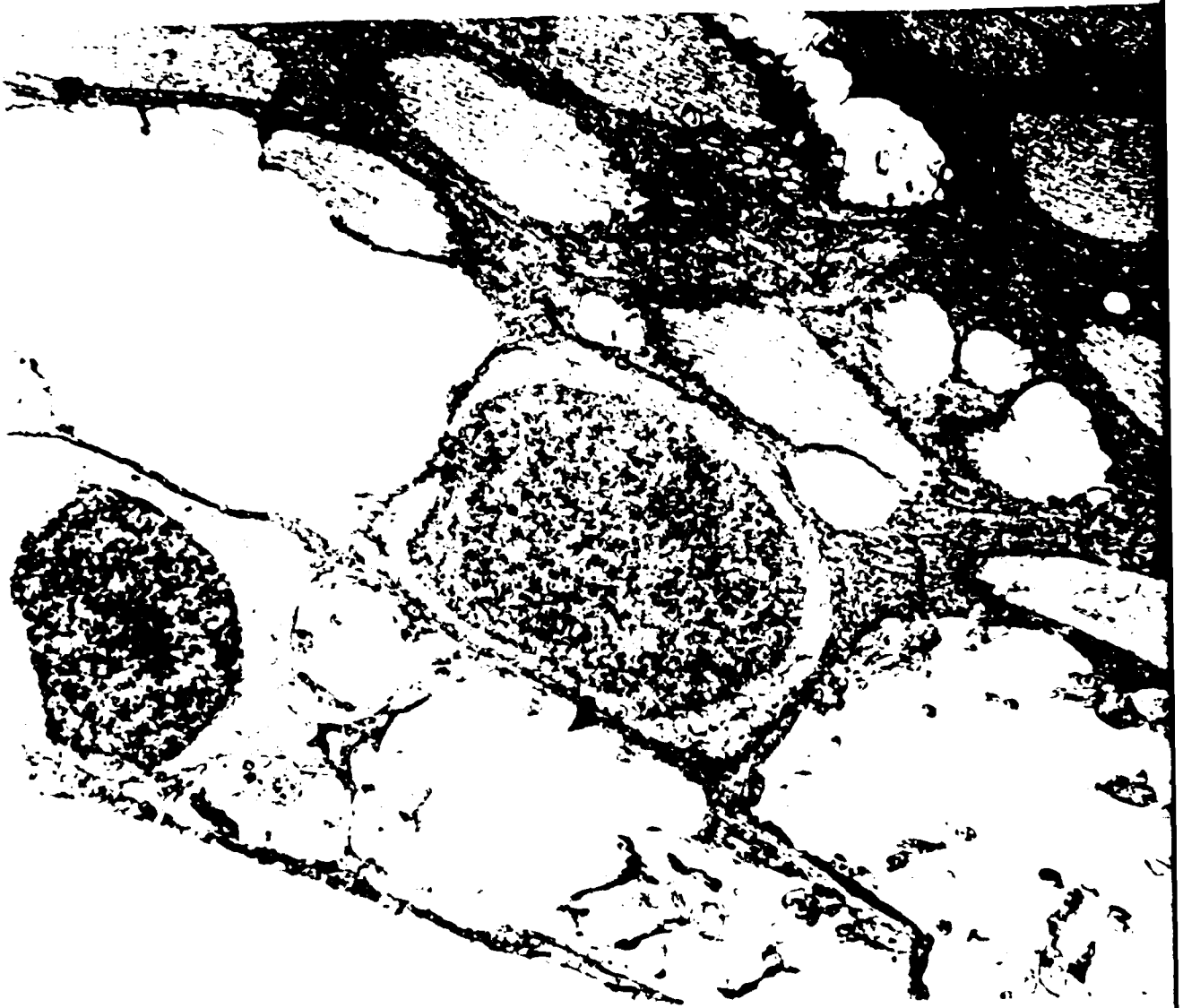


Figure 32 [illegible] Left Eye One week lesion

In the foveola region, clear spaces are noted along the inner limiting membrane. These may represent degenerating nerve fibers or be a result of fixation artifact. They are limited to a very small area of retina (see figures 32 A and B). x 7500

END

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